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# Results of Shipboard Approval Tests of Ballast Water Treatment Systems in Freshwater

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Homeland Security

# N O T I C E

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16. Abstract (MAXIMUM 200 WORDS)

The U.S. Coast Guard Research and Development Center (USCG RDC) tasked the Great Ships Initiative (GSI) with implementing the United States Environmental Protection Agency, Environmental Technology Verification (ETV) Program's *Draft Generic Protocol for the Verification of Ballast Water Treatment Technology in Shipboard Installations, version 5.2,* (ETV DSP) on board an operating commercial vessel to identify areas of possible improvement. ETV DSP implementation included use of a prototype Shipboard Filter Skid (p3SFS), which the ETV DSP incorporates as an optional sampling approach. A secondary objective of *Project 41012* was to evaluate, on a limited basis, the biological efficacy and environmental soundness of a prototype ballast water management system (BWMS). Four test cycles took place on board a Great Lakes self-unloading bulk freighter, the Motor Vessel *Indiana Harbor*, with the prototype BWMS active during two of them. Overall GSI found both the ETV DSP and p3SFS to be feasible and promising approaches to shipboard validation of prospective BWMSs, and identified specific ways to improve them. The prototype BWMS operated during these tests reduced the densities of live plankton in the *Indiana Harbor*'s ballast tanks, but the treated discharges did not meet International Maritime Organization (IMO) standards in these tests.

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# EXECUTIVE SUMMARY

This United States Coast Guard Research and Development Center (USCG RDC) Technical Report (TR) fulfills Great Ships Initiative (GSI) contract implementation of USCG RDC Project No. 41012 titled *Shipboard Approval Tests of Ballast Water Treatment Systems in Freshwaters* (hereafter, *Project 41012)*. Specifically, this TR presents GSI methods, results, conclusions and recommendations in its role of implementing, and identifying areas of improvement in, the United States Environmental Protection Agency (USEPA) Environmental Technology Verification (ETV) Program's *Draft Generic Protocol for the Verification of Ballast Water Treatment Technology in Shipboard Installations, version 5.2*, (hereafter, ETV DSP) (USEPA, 2012). The first objective of the ETV DSP demonstration and review exercise was implementation of the ETV DSP on an operating commercial vessel, including a skid-mounted sampling system, known as the prototype 3 Shipboard Filter Skid (p3SFS), developed by the Naval Research Laboratory (NRL) in Key West, Florida, which the ETV DSP incorporates as an optional sampling approach. A secondary objective of *Project 41012* was to evaluate, on a limited basis, the biological treatment efficacy and environmental soundness of a prototype ballast water management system (BWMS).

GSI's evaluation of the ETV DSP and p3SFS took place during four test cycles (TCs) on board a Great Lakes self-unloading bulk freighter, the Motor Vessel (M/V) *Indiana Harbor* (IH). During two test cycles, a partial and temporary NaOH prototype BWMS also was activated. Intake sampling occurred during IH ballasting at a southern Lake Michigan or southeastern Lake Erie port. Discharge sampling occurred during IH deballasting operations in western Lake Superior.

GSI implemented each of the four TCs following a Test/Quality Assurance Plan (TQAP), which itself was consistent with the ETV DSP. All TCs generally met ETV DSP physical/chemical and biological validity requirements; though there were some potentially relevant inconsistencies which are noted in this report.

The GSI team found both the ETV DSP and p3SFS to be feasible and promising approaches to shipboard validation of prospective BWMSs, but identified specific ways to improve them. For the ETV DSP, these include:

- Requiring test organizations (TOs) to explicitly define in the TQAP how they will protect personnel health and safety through preventing exposure to harmful substances and organisms in ballast water, and overextension of staff;
- Requiring acceptable limits for sampling to be considered proportional so that at a minimum the TO can make a *post facto* determination of validity;
- Requiring that TOs provide evidence, from the literature or from new empirical tests, to eliminate intake water toxicity as a source of BWMS discharge toxicity; and
- Removing requirements for meeting intake water chemistry challenge water target conditions, lowering the presumed percent live for the $\geq 50\ \mu m$ size class of organisms in preserved intake samples, and allowing a higher presumption only with seasonal validation.

For the p3SFS, these include:

- Providing enough sample ports if a vessel requires multiple sampling locations such that vessel crews do not need to move the ports during testing;

- Installing a second pressure sensor downstream of the canister so that the differential pressure across the canister can be measured more reliably than with the differential pressure sensor;
- Switching the p3SFS pump to a self-priming model to expand the range of conditions in which the sampling system can operate;
- Modifying the p3SFS to allow collection of discrete grab samples and for collection of two drip samples simultaneously into two 19 L carboys;
- Adding alarms, including to indicate overly high or low sample flow; and
- Conducting validation experiments to determine the most accurate inline sensors to measure temperature and turbidity, as well as, the data output type for the p3SFS that produces the most accurate and reliable results; and
- Based on post shipboard validations at the GSI land-based facility of the p3SFS performance (Appendix A), installing the sample flow meter in a straight length of pipe long enough to ensure accurate readings.

The secondary objective of this project, GSI's assessment of the prototype NaOH BWMS's performance against the USCG's *Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters* (USCG, 2012) using the ETV DSP (with necessary deviations), successfully produced a partial assessment of the BWMS's performance in the context of the ETV DSP. The zooplankton analysis alone was unsuccessful due to interference issues associated with the p3SFS flow meter and flow control apparatus (detected only in follow-up validation exercises at the GSI land-based facility). The GSI team was able to complete all sampling and necessary biological efficacy analyses, however, consistent with the ETV DSP. Valid results pertaining to densities of live organisms ≥ 10 $\mu$m and < 50 $\mu$m in minimum dimension in treatment discharge showed the BWMS's discharge were two orders of magnitude above the USCG's standard. Concentrations of regulated organisms < 10 $\mu$m in minimum dimension (*E. coli* and *Enterococcus spp*.) were already below the discharge limit upon intake. No trihalomethanes, haloacetic acids, or bromate ions were detected in the treatment discharge samples. However, measurable concentrations of sodium ion were found in the treatment discharge from tanks 3P and 4P in both TCs where the prototype BWMS was activated. Whole Effluent Toxicity (WET) tests conducted according to protocols described here showed a significant reduction in the cladoceran *Ceriodaphnia dubia* reproduction exposed to treated effluent and dilutions thereof, relative to controls. No reproduction effects were detected in any other test organism, and no acute effects were detected.

The report concludes the ETV DSP represents a strong starting point for a standard shipboard BWMS verification protocol, but greater specificity and clarity in specific areas are needed to assure that TOs have sufficient guidance to avoid expensive false starts or compromised outcomes. For example, the ETV DSP should provide guidance for: protecting TO staff health and safety during shipboard tests; unplanned changes to ballast flow rates; sample proportionality; and whether "whole tanks" need to be sampled on discharge or whether partial tanks are valid sources of discharge water. Given resident toxicity of many harbors, GSI also recommends that the ETV DSP require a qualitative determination for WET of intake water, and perhaps allow greater flexibility around valid threshold conditions. In particular, particulate organic matter (POM) and particulate organic carbon (POC) requirements are more easily and thoroughly addressed in land-based testing. In terms of the p3SFS, GSI recommends relocation of the p3SFS flow meter to a length of pipe free of upstream obstructions; provision of additional sample ports; improved filter sock construction; enhanced drip and grab sample collection capacity; more accurate temperature and turbidity detection capability; digital card error reporting; and improved pause and resume capacity. The user-interface would be improved by revised alarms, better p3SFS "cleanability" and guidance, a trend screen, installation checklists, and a flow-rate display.

# TABLE OF CONTENTS

# TABLE OF CONTENTS (Continued)

# LIST OF FIGURES

# LIST OF FIGURES (Continued)

# LIST OF TABLES

## LIST OF TABLES (Continued)

# LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS

| | |
|---|---|
| %D | Percent Difference |
| %T | Percent Transmittance |
| µM | Micrometer |
| AOC | Area of Concern |
| ASC | American Steamship Company |
| BWMS | Ballast Water Management System |
| BWT | Ballast Water Treatment |
| CFD | Computational Fluid Dynamics |
| CFU | Colony Forming Unit |
| CMFDA | 5-Chloromethylfluorescein Diacetate |
| $CO_2$ | Carbon Dioxide |
| COC | Chain of Custody |
| DI | Deionized |
| DO | Dissolved Oxygen |
| DOC | Dissolved Organic Carbon |
| DOM | Dissolved Organic Matter |
| DQO | Data Quality Objective |
| DSP | Draft Shipboard Protocol |
| DST | Defined Substrate Technology |
| ETV | Environmental Technology Verification |
| FDA | Fluorescein Diacetate |
| FH | Filter Housing |
| Ft. | Feet |
| GSI | Great Ships Initiative |
| HCl | Hydrochloric Acid |
| HDPE | High Density Polyethylene |
| HMI | Human Machine Interface |
| HPC | Heterotrophic Plate Counts |
| HPCA | Heterotrophic Plate Count Agar |
| ID | Internal Diameter |
| IH | M/V *Indiana Harbor* |
| IMO | International Maritime Organization |
| IRNP | Isle Royale National Park |
| ITR | Interim Technical Report |
| LAN | Local Area Network |
| LOQ | Limit of Quantification |
| LSRI | Lake Superior Research Institute |
| M/V | Motor Vessel |
| MARAD | United State Maritime Administration |
| MDL | Method Detection Limit |
| MM | Mineral Matter |
| MPN | Most Probable Number |

## LIST OF ACRONYMS, ABBREVIATIONS, AND SYMBOLS (Continued)

| | |
|---|---|
| NaOH | Sodium Hydroxide |
| ND | University of Notre Dame |
| NEMWI | Northeast Midwest Institute |
| NPOC | Non-Purgeable Organic Carbon |
| NRL | Naval Research Laboratory |
| NRRI | Natural Resources Research Institute |
| p3SFS | Prototype 3 Shipboard Filter Skid |
| PI | Principal Investigator |
| PLC | Programmable Logic Controller |
| POC | Particulate Organic Carbon |
| POM | Particulate Organic Matter |
| PP | Polypropylene |
| PPE | Personal Protective Equipment |
| PSC | Percent Similarity |
| QA | Quality Assurance |
| QA/QC | Quality Assurance/Quality Control |
| QAPP | Quality Assurance Project Plan |
| QC | Quality Control |
| RDC | Research and Development Center |
| RDTE | Research, Development, Testing, and Evaluation |
| RPD | Relative Percent Difference |
| SD | Secure Digital |
| SOP | Standard Operating Procedure |
| SOW | Scope of Work |
| SBW | Sterile Ballast Water |
| TC | Test Cycle |
| TO | Testing Organization |
| TOC | Total Organic Carbon |
| TQAP | Test/Quality Assurance Plan |
| TR | Technical Report |
| TSS | Total Suspended Solids |
| TVE | Tank Volume Equivalent |
| UMD | University of Minnesota-Duluth |
| US GPM | United States Gallons per Minute |
| USCG RDC | United States Coast Guard Research and Development Center |
| USCG | United States Coast Guard |
| USEPA | United States Environmental Protection Agency |
| UV | Ultraviolet |
| UWS | University of Wisconsin-Superior |
| VO | Verification Organization |
| WET | Whole Effluent Toxicity |
| YSI | Yellow Springs Instruments |

# 1 INTRODUCTION AND BACKGROUND

## 1.1 Overview and Objectives

This United States Coast Guard Research and Development Center (USCG RDC) Technical Report (TR) presents methods, results, conclusions and recommendations relative to Test Cycles (TCs) 1 through 4 of the USCG RDC Project No. 41012 titled *Shipboard Approval Tests of Ballast Water Treatment Systems in Freshwaters*, hereafter referred to as *Project 41012*. The two primary objectives of *Project 41012* were to:

I. Implement and identify areas of improvement to the United States Environmental Protection Agency (USEPA) Environmental Technology Verification (ETV) Program's *Draft Generic Protocol for the Verification of Ballast Water Treatment Technology in Shipboard Installations, version 5.2*, hereafter referred to as ETV DSP (USEPA, 2012), to improve its effectiveness for verification of the biological treatment efficacy and environmental acceptability of a ballast water management system (BWMS) on an operating cargo ship; and
II. Implement in fresh water a skid-mounted sampling system, known as the prototype 3 Shipboard Filter Skid (p3SFS), developed by the Naval Research Laboratory (NRL) in Key West, Florida, which the ETV DSP incorporates as an optional sampling approach, and identify areas of possible improvement.

A secondary objective of *Project 41012* was to evaluate, on a limited basis, the biological treatment efficacy and environmental soundness of a prototype BWMS being developed by the United States Geological Survey (USGS) and others that utilizes sodium hydroxide (NaOH) treatment to a high pH followed by carbon dioxide ($CO_2$) neutralization to pH 6.0 to 8.8.

The four TCs of *Project 41012* took place onboard the Great Lakes self-unloading bulk freighter Motor Vessel (M/V) *Indiana Harbor* (IH). The IH, operated by the American Steamship Company (ASC), is a 305 meter bulk freighter that travels exclusively in the upper four Great Lakes. The vessel has 18 ballast tanks, including forepeak and aftpeak tanks, and a total ballast capacity of 62,166 $m^3$. The NaOH BWMS functioned as a partial and temporary installation onboard the IH during TCs 2 and 3 only.

In keeping with the ETV DSP, the four TCs took place following separate Test/Quality Assurance Plans (TQAPs; GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b). Intake sampling occurred during IH ballasting operations either at a port located in southern or central Lake Michigan (TCs 1-3) or a port located in southeastern Lake Erie (TC4 only). Discharge sampling occurred during IH deballasting operations at ports located in western Lake Superior.

During TCs 1-3, ballast intake and discharge samples were collected from up to three experimental ballast tanks located on the port side of the IH (Table 1). During TC2 and TC3, the NaOH BWMS was active. Two ballast tanks were treated and one tank was untreated. The untreated tank was referred to as a "mock treatment" tank, as the ETV DSP requires whole-ship treatment and the untreated tank needed to be handled as though it were treated for purposes of validating the ETV DSP (Table 1). During TC4, which occurred during an atypical IH ballast operation, i.e., the vessel did not ballast on a tank by tank basis; up to three ballast tank volume-equivalent (TVE) samples were collected during both intake and discharge operations irrespective of any association with specific ballast tanks (Table 1).

Table 1. *Project 41012* Sampling Arrangement: Experimental ballast tanks.

| Test Cycle (TC) | Ballast Tank Sampling Arrangement | |
|---|---|---|
| | Intake | Discharge |
| 1 | 5P, 2P, 3P | 2P, 3P, 5P |
| 2 | 5P, 2P | 3P (treated), 4P (treated), 5P (mock treatment) |
| 3 | 5P, 2P | 3P (treated), 4P (treated), 5P (mock treatment) |
| 4 | Three ballast “tank volume-equivalent” (TVE) samples | Two ballast “tank volume-equivalent” (TVE) samples |

## 1.2 Roles and Responsibilities of Organizations

*Project 41012* involved several organizations with responsibilities divided among them. These organizations include the Testing Organization (TO), BWMS Developer, ship operator, Verification Organization (VO), federal partners and external collaborators. The fundamental roles and responsibilities of these organizations were consistent throughout all four TCs of *Project 41012*, with the exception of external collaborators who were involved only during TC3.

### 1.2.1 Testing Organization

The TO, GSI, was responsible for preparing the TC-specific TQAPs (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b), which also included GSI’s Shipboard Quality Assurance Project Plan (QAPP; GSI, 2013c) as an appendix, and for working with the VO (USCG RDC) to assure approval of the TQAPs. GSI was responsible for conducting the testing; for managing, evaluating and reporting on all data generated during the testing; and for preparing, circulating for comment to the VO and BWMS Developer and producing and finalizing the Interim Technical Reports (ITRs; GSI, 2012c; GSI, 2013d; GSI, 2013e; GSI, 2013f) and this TR. GSI also was responsible for coordinating with ASC’s shore-side and shipboard engineering staff to facilitate and oversee TQAP implementation coordination with shipboard operations. Finally, GSI was responsible for maintaining the security and safety of GSI personnel during test activities.

### 1.2.2 Ballast Water Management System Developer

The BWMS Developer, consisting of researchers from the USGS Leetown Science Center and the Isle Royale National Park (IRNP), in collaboration with the ship operator (ASC), was responsible for installation and commissioning of the prototype NaOH BWMS onboard the IH and training of the vessel’s crew on operation of the system. The BWMS Developer was also responsible for confirming that the BWMS was operating correctly prior to biological treatment efficacy testing and assuring that treated ballast water was fully neutralized and safe for discharge to the receiving system prior to deballasting. The BWMS Developer was also responsible for providing the TO, ship operator and VO with all necessary information, including operation and maintenance manuals, and for making decisions on behalf of the BWMS Developer during implementation of the TC2 and TC3 TQAPs (GSI, 2012b; GSI, 2013a). In addition, the BWMS Developer was responsible for making a representative available for logistical and technical support, as required. The BWMS Developer also reviewed the TC2 and TC3 ITRs (GSI, 2013d; GSI, 2013e), with the understanding that these documents did not constitute an ETV evaluation or regulatory approval.

#### 1.2.3 Ship Operator

The ship operator, ASC, was responsible for working with the GSI Test Manager to schedule and organize logistics associated with the testing. ASC was also responsible for notifying GSI of any logistical or operational developments that could affect the *Project 41012* testing process and/or results and for ensuring proper installation and operation of the BWMS onboard the IH, including preparation of sample ports and neutralization of the treated discharge prior to deballasting (relevant to TCs 2 and 3 only). ASC was also responsible for ensuring that IH ballast operations (i.e., location, holding time, sampling, etc.) were consistent with those criteria detailed in the TC-specific TQAPs (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b).

#### 1.2.4 Verification Organization

The VO, USCG RDC, was responsible for reviewing and approving the TC-specific TQAPs (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b), and ITRs (GSI, 2012c; GSI, 2013d; GSI, 2013e; GSI, 2013f), and this TR. The VO also received and reviewed periodic progress reports and other relevant *Project 41012* documents. In addition, the VO was responsible for collaborating with GSI and the United States Maritime Organization (MARAD) to administer testing activities on board the IH; USEPA ETV personnel to provide *Project 41012* updates; and participating in conferences/discussions of TC implementation, results, and suggested changes.

#### 1.2.5 Federal Partners

The MARAD Project Officer and USCG RDC Project Manager were responsible for obtaining federal partner reviews of the TC-specific TQAPs (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b), TC-specific ITRs (GSI, 2012c; GSI, 2013d; GSI, 2013e; GSI, 2013f) and this TR.

#### 1.2.6 External Collaborators

During TC3, personnel from the University of Notre Dame (ND), and Yellow Springs Instruments (YSI) obtained subsamples from the TO for independent research on automated and/or expedited detection and enumeration methodologies.

### 1.3 Purpose and Features of the Environmental Technology Verification Program's Draft Shipboard Protocol

The USCG RDC tasked GSI with implementing the ETV DSP (USEPA, 2012) and identifying areas of improvement through a series of four TCs undertaken on board a commercial cargo ship operating solely in the Great Lakes. The ETV DSP, under development by the USEPA ETV Program and several federal and non-governmental partners, provides guidance to TOs on the necessary elements of shipboard BWMS verification tests. These include technology acceptability criteria, BWMS specifications and information, TQAP content requirements, experimental design requirements, sampling and analysis procedures, quality assurance/quality control (QA/QC) and data management and reporting (USEPA, 2012). Most importantly, the ETV DSP guides TOs in evaluating the performance characteristics of commercial-ready BWMS technologies with regard to three verification factors: Biological Treatment Efficacy, Environmental Acceptability and Operational Performance. *Project 41012's* scope encompassed evaluation of the ETV DSP relative to biological treatment efficacy and environmental acceptability only. In order to achieve this evaluation using the ship and BWMS of opportunity, several deviations to the ETV DSP were deemed necessary and acceptable by the TO and VO. These deviations are summarized in Table 2.

Table 2. Summary of deviations made to the U.S. Environmental Protection Agency, Environmental Technology Verification Program's Draft Shipboard Protocol (ETV DSP; USEPA 2012) during implementation of *Project 41012*.

| Description of Deviation | Brief Explanation of Deviation | Reason (Root Cause) for Deviation | Description of Impact on the Experiment |
|---|---|---|---|
| Test Objective | Verification testing of the subject BWMS was the objective of the ETV DSP, but the primary objective of *Project 41012* was to implement and assess the ETV DSP itself. | The Scope of Work (SOW) for *Project 41012* specified that the primary objective was to implement and identify areas of improvement in the ETV DSP. | Minimal. *Project 41012* generated limited information about the biological treatment efficacy and environmental acceptability of the prototype NaOH BWMS. |
| Insufficient Treatment Tanks | There were insufficient ballast tanks subject to treatment during the course of the *Project 41012* to allow an adequate volume of sample water to be analyzed consistent with ETV DSP requirements for sample volume and integrity during analysis. | Temporary and partial nature of the prototype BWMS. | Minimal. The prototype BWMS was capable of treating water only in experimental tanks 3P and 4P, but discharge sampling was necessary for at least three experimental tanks to assure adequate time for analysis of sample volumes required. Therefore, *Project 41012* refers to untreated tanks 2P and 5P as additional untreated experimental tanks, or "mock treatment" (i.e., standing in for treatment) tanks. |
| Pre-Treatment Samples Not Collected on Intake | The inline NaOH injection port was installed too close to the p3SFS intake sample port for GSI to safely sample pre-treatment water (Figure 1). | The proximity of the p3SFS intake sample port to the inline NaOH injection port created a safety concern; any interruption in main ballast flow could have caused NaOH treated water to be taken up into the intake sample port and increased the pH of the pre-treatment sample water above a level safe for handling. The same scenario would also have caused organisms retained in the p3SFS to be dosed with a high concentration of NaOH thereby invalidating the sample. | Minimal. The TC2 and TC3 TQAPs called for collection of representative, continuous, in-line samples of ballast intake to designated untreated,(i.e., mock-treatment), tanks 2P and 5P, neither of which received treatment, but which were filled at a time similar enough to the treatment tanks for the samples to reflect challenge conditions for the "true" treatment tanks 3P and 4P. |
| Scope of Biological Treatment Efficacy Evaluation | The ETV DSP calls for a single TQAP and Verification Report to cover the entire series TCs within a given BWMS evaluation. For purposes of *Project 41012*, each TC was a stand-alone assessment of the ETV DSP with a distinct TQAP and ITR. | The SOW for *Project 41012* specified that a separate TQAP and ITR be generated for each TC, and that a final TR describe the implementation of all four TCs and their outcomes. | Minimal. Separate TQAPs and ITRs were developed for each specific TC. This TR summarizes the data and findings from all four TCs. |
| Partial Installation of BWMS | The ETV DSP requires that tests be performed on a permanent, whole-ship commercial ready BWMS. The NaOH BWMS is not a commercially-ready system, and is a partial, temporary installation. | The vessel operator, ASC, did not want to invest in a whole ship installation until it was certain the BWMS would function effectively; a multi-year process. | Minimal. The partial BWMS installation requires flushing between every ballast tank operation on discharge. In addition, the same experimental treatment tanks were used in all four TCs. |

Table 2. Summary of deviations made to the U.S. Environmental Protection Agency, Environmental Technology Verification Program's Draft Shipboard Protocol (ETV DSP; USEPA 2012) during implementation of *Project 41012* (Continued).

| Description of Deviation | Brief Explanation of Deviation | Reason (Root Cause) for Deviation | Description of Impact on the Experiment |
|---|---|---|---|
| Operation of the BWMS | The ETV DSP requires that the vessel's crew operate the BWMS, and requires it to be operated continuously during a ≥ 1 year testing period. During *Project 41012*, the NaOH BWMS was operated by the BWMS Developer and the system was operated only during TCs 2 and 3 of the four TCs. | The NaOH BWMS is not commercially-ready and could not be operated by the ship's crew. | Minimal. Analyses of operational, safety, reliability, and cost of BWMS operation were not conducted because it was not in the purview of *Project 41012*. |
| Technical Report (TR) Deliverable | The ETV DSP requires a Verification Report of the test results from five TCs conducted over ≥ 1 year period. During *Project 41012*, an ITR detailing the results and findings from each TC was drafted, with this TR developed to summarize *Project 41012* results across all four TCs. | *Project 41012* deliverables required each TC to have its own TQAP and ITR. | Minimal. Four separate TQAPs and ITRs were developed for each of the four TCs. This TR summarizes the data from all four TCs and is consistent with the format provided to GSI by the VO. |
| Continuous, *In Situ* Water Quality Data Collected for Temperature and Turbidity Only | The ETV DSP specifies that *in situ*, continuous measurements be made for the following core water quality parameters: temperature, pH and chlorophyll *a* plus the auxiliary parameter turbidity. During *Project 41012*, only temperature and turbidity were measured continuously *in situ*. | The NRL p3SFS has been developed to measure temperature and turbidity only using the *in situ*, continuous approach. | Minimal. Discrete measurement data were available for pH and total chlorophyll (rather than chlorophyll *a*). |
| Lack of *In Situ* Flow Monitoring | The ETV DSP specifies that *in situ*, continuous measurements be made for ballast system flow rate. During TCs 1-4, ballast system flow rate was not measured. | For TCs 1-4, the magnetic flux flow meter was not installed, or correctly wired to the pSFS3. | Minimal. In lieu of *in situ*, continuous flow monitoring, GSI recorded tank heights every five to ten minutes to approximate flow rates in the ballast main. |
| Integrated Samples Collected for Water Quality rather than Grab Samples | The ETV DSP specifies that the following water quality samples be collected in triplicate as discrete grab samples: total suspended solids (TSS), particulate organic matter (POM) and dissolved organic matter (DOM). | The p3SFS does not permit the collection of grab samples. These samples were collected from the time-integrated drip sample. | Minimal. Samples for TSS, POM and DOM were still collected and analyzed. |

Table 2. Summary of deviations made to the U.S. Environmental Protection Agency, Environmental Technology Verification Program's Draft Shipboard Protocol (ETV DSP; USEPA 2012) during implementation of *Project 41012* (Continued).

| Description of Deviation | Brief Explanation of Deviation | Reason (Root Cause) for Deviation | Description of Impact on the Experiment |
|---|---|---|---|
| Use of Single Vital Stain and Extended Length of Analysis Time | The ETV DSP specifies that a combination of two vital stains, Fluorescein Diacetate (FDA) and 5-Chloromethylfluorescein Diacetate, be used for analysis of organisms in the ≥ 10 and < 50 $\mu$m size class and that samples be examined for a maximum of 20 min. For *Project 41012*, this size class was stained using FDA only. | The GSI standard operating procedure (SOP) for this size class specifies the use of FDA only and samples are examined for up to 90 minutes. | Minimal. GSI, per ETV DSP requirements, split the treatment discharge samples in half and heat killed one half to determine the false positive error rate. |

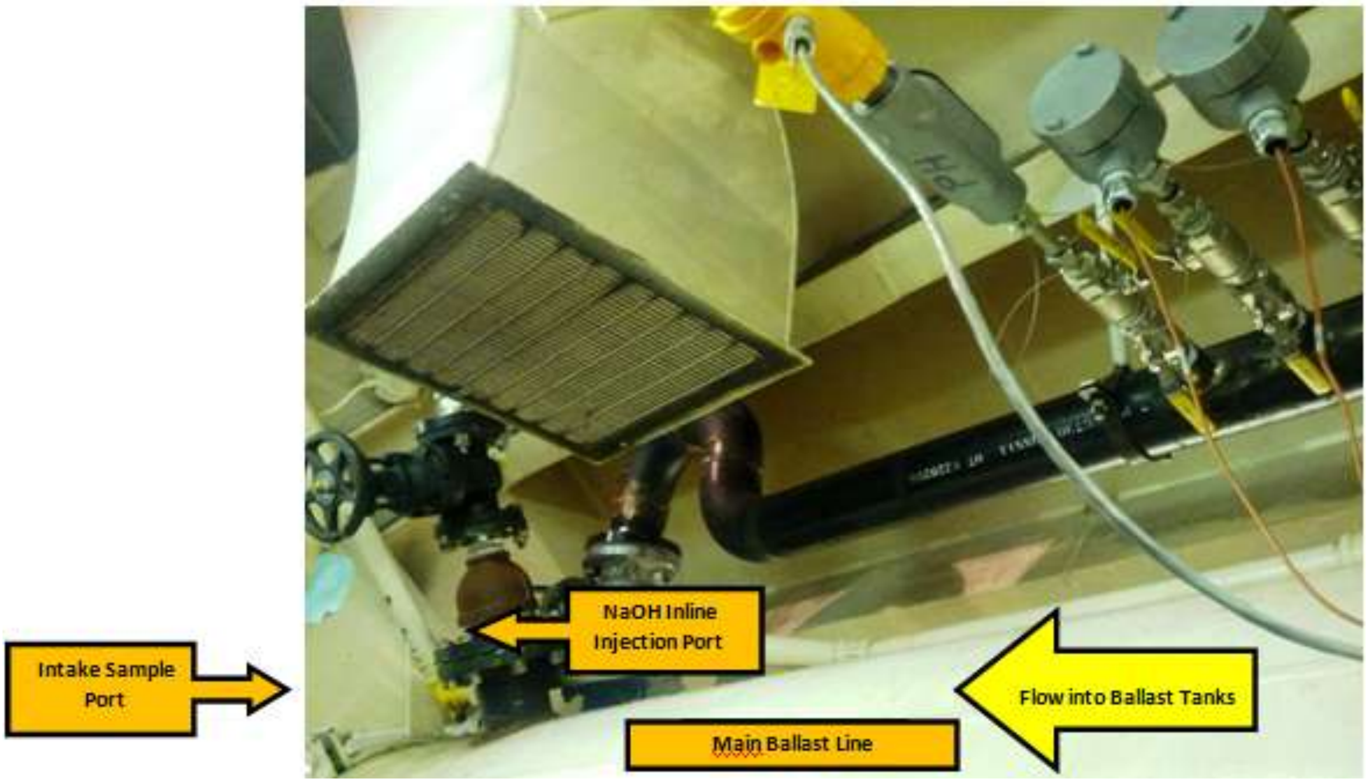


Figure 1. Location of the ballast water management system's sodium hydroxide inline injection point relative to the GSI intake sample port.

## 1.4 Description of the Test Vessel

The IH is operated by ASC of Williamsville, New York (Table 3). The IH was built in 1979 and is a self-unloading bulk freighter that plies exclusively in the upper four Great Lakes in long-haul transport of iron ore pellets and western coal. The IH is 305 m in length with a breadth of 32 m and depth of 17 m. It travels at an average full speed of 13 knots (24 km/hr) and is powered by four 3500 HP General Motors Electro Motive Division diesel engines. There are seven cargo holds onboard with 37 hatches. The vessel's engine room is Automated Control System Certified and her crew complement is 21. The IH's ballast system comprises 18 ballast tanks including forepeak and aftpeak tanks, with a total ballast capacity of 62,166 $m^3$. The ship has four ballast pumps of 2,952 $m^3$/hr each, for a combined total flow rate of 11,808 $m^3$/hr. GSI oversaw installation on the IH of a magnetic flux flow meter in the ballast main to help assure proportional sampling for the test, but it was not in place for TCs 1-3.

Table 3. Test vessel data and service description.

| Vessel Data | |
|---|---|
| Name | M/V *Indiana Harbor* |
| IMO # and/or CG VIN | IMO #7514701, CG Official #610401 |
| Owner | U.S. Bank National Association, 1 Federal Street, 3rd Floor, Boston, MA 02110 |
| Operator | American Steamship Company, 500 Essjay Road, Williamsville, NY 14221 |
| Service Description | |
| Route and Ports Served | Various; exclusively within the Great Lakes (U.S. & Canada). Typically loading cargo in western Lake Superior (i.e., the Port of Two Harbors or the Port of Duluth-Superior) and unloading cargo in lakes Michigan or Erie (i.e., Indiana Harbor, Detroit, Ashtabula or Muskegon) |
| Average Voyage Duration and Frequency | 5 to 6 Days per voyage; approx. 50 voyages per year |
| Annual Operating Schedule | Approximately late March until early January annually |

## 1.5 Description of the Ballast Water Management System

The prototype NaOH BWMS was active only during TCs 2 and 3 with the treatment process identical except that the target pH was 11.5 for TC2, while for TC3 it was 12.0 (Table 4). In addition, the two treatment ballast tanks 3P and 4P were not cleaned prior to TC2 but were cleaned prior to TC3. Finally, prior to TC3, the BWMS Developer deemed the ship's 76.2 cm ballast line (volume of 181,700 L) a likely source of contamination in the context of partial installation. In order to address this issue during TC3, the BWMS Developer connected the port and starboard forward and aft ballast lines using the impeller pump from the NaOH dosing system to create a treatment loop through the lines. NaOH was added to the line using the same venturi system for dosing the tanks and held for most of the ship's voyage. Neutralization of the line occurred prior to the ballast tanks.

The prototype NaOH BWMS process involved five steps:

1. Volume calculation, based on previous analyses of NaOH demand of the test waters and sediments, of 30 % (w/v; TC2) or 50 % (w/v; TC3) NaOH necessary to raise the pH of the ballast water from ambient, i.e., near neutral, to a target level, e.g., pH 11.5 or 12.0;
2. In-line injection of the calculated volume of 30 % (w/v) or 50 % (w/v) NaOH during ballast intake;
3. Treated ballast water retention (i.e., while the IH was in transit);

4. Neutralization of the treated ballast water with $CO_2$ injected into treated tanks; and
5. Verification of complete neutralization, i.e., pH 6 to 8.8, prior to ballast discharge.

The partial and temporary BWMS installation tested as part of *Project 41012* comprised an in-line dosing system to inject 30 % (w/v) NaOH during TC2 and 50 % (w/v) NaOH during TC3 into ballast water destined for tanks 3P and 4P, and an in-tank dispersal system to distribute shore-positioned $CO_2$ gas through the treated ballast water prior to discharge. Over time, the BWMS design will integrate the $CO_2$ source as part of the onboard system, possibly employing stack emissions.

The 30 % (w/v) or 50 % (w/v) NaOH was stored on the IH's deck in temporary temperature-controlled holding tanks. A series of valves, flow meters, pressure gauges and a programmable logic controller (PLC) regulated the flow of NaOH into a venturi to assure that the ballast was dosed with a target mass of NaOH. A high pressure water line introduced the treated water into the main ballast line header in the engine room and then into the two designated treatment ballast tanks. NaOH loading occurred during approximately 30 minutes followed by a rinse of the remaining water entering the two ballast tanks. Both tanks branch off of the ballast main header with similar downward-facing bell mouths on the interior bottom tank surface. When the target volume was reached, the data logger closed the three-way valve to flush the venturi injector with water. Multiple conductivity/pH meters downstream of the mixing point confirmed reagent flow. A data logger recorded a running total of 30 % (w/v) or 50 % (w/v) NaOH injected (data not available to GSI).

Ballast tank 3P was equipped with fifteen discrete water quality sampling points inside the tank and tank 4P was equipped with eight. The sample tubing used in these ballast tanks was 1.9 cm clear PVC. Each sampling tube ran from its selected position to isolation valves with steel pipe nipples that extended through a single steel plate bolted to the bulkhead between the tank and the conveyor tunnel. During testing operations, ballast water gravity flowed through each in-tank sampling tube, on demand, to a single sampling valve mounted outside each tank in the conveyor tunnel. The BWMS Developer monitored and documented, from the IH control room, pH and conductivity data from two conductivity and one pH wet tap probes (Signet type) located in each treated tank and the ballast line (data not available to GSI).

Table 4. Technical specifications of the prototype sodium hydroxide ballast water management system during Test Cycles 2 and 3.

| Test Cycle | BWMS Treatment | Retention Time | Neutralization Time | Post-Neutralization | Process for Confirmation of Successful Neutralization |
|---|---|---|---|---|---|
| 2 | Target: 11.5; Actual: Data not provided to GSI | 2 days, 12 hours | Tank 3P: 3+ hours; Tank 4P: 3 hours | Target: 6.0 – 8.0; Actual: Data not provided to GSI | The BWMS Developer's representative confirmed successful neutralization prior to ballast discharge on 21 October 2012 by completing and signing GSI FORM: *Ballast Water Management System Neutralization Verification.* |
| 3 | Target: 12.0; Actual: 11.7 | 3 days, 10 hours | Tank 3P took longer to neutralize than Tank 4P. Neutralization times not provided to GSI. | Target: 6.0 – 8.0; Actual: Data not provided to GSI | The BWMS Developer's representative confirmed successful neutralization prior to ballast discharge on 16 August 2013 by completing and signing GSI FORM: *Ballast Water Management System Neutralization Verification.* |

# 2 EXPERIMENTAL DESIGN

## 2.1 Overview and Calendar

The experimental objective of *Project 41012* was to implement the ETV DSP, including the p3SFS sampling approach, in the context of four TCs conducted onboard the IH to generate recommendations for improvement, and/or implementation guidelines. For each TC, the TO developed and implemented individual TQAPs (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b) and submitted ITRs to the VO (GSI, 2012c; GSI, 2013d; GSI, 2013e; GSI, 2013f) with recommendations for ETV DSP improvement. Table 5 summarizes the overall sequence of *Project 41012* testing, evaluation and reporting activities.

Intake sampling always occurred during normal IH ballasting operations either at a port located in Lake Michigan (TCs 1–3; Table 5) or a port in southeastern Lake Erie (TC4; Table 5). All discharge sampling occurred during normal IH deballasting operations in western Lake Superior (Table 5). During TCs 1 through 3, ballast intake and discharge samples were collected from up to three experimental ballast tanks located on the port side of the IH (Table 5). During TC4, owing to an atypical IH ballast operation that the vessel did not ballast on a tank by tank basis, up to three ballast (TVE) samples were collected during both intake and discharge operations irrespective of any association with specific ballast tanks (Table 5). In lieu of *in situ*, continuous ballast flow monitoring, GSI personnel recorded the rate of change in tank heights (based on tank height observations every five to ten minutes) and associated the information with tank volume to approximate flow rates in the ballast main. This information was then contrasted with recorded sample flow rates to determine proportionality with recorded p3SFS sample flow rates.

Table 5. Calendar of testing, evaluation, and reporting for Test Cycles 1-4 of *Project 41012*.

<table>
<tr><th>Test Cycle</th><th>Date</th><th colspan="4">Project Activity</th></tr>
<tr><td rowspan="10">1</td><td>May 24, 2012 - July 23, 2012</td><td colspan="4">Test/Quality Assurance Plan (TQAP) development, review and finalization</td></tr>
<tr><td rowspan="2">July 25, 2012</td><td colspan="4">M/V Indiana Harbor ballast intake sampling at the Port of Indiana Harbor, Hammond, Indiana, in southern Lake Michigan: TEST CODE: 12-ETV-1F</td></tr>
<tr><td>Tank 5P intake sampling:<br>15:35 to 17:09</td><td colspan="2">Tank 2P intake sampling:<br>17:58 to 19:30</td><td>Tank 3P intake sampling:<br>21:37 to 23:08</td></tr>
<tr><td>July 26, 2012 – July 29, 2012</td><td colspan="4">M/V Indiana Harbor voyage to Port of Superior, Wisconsin</td></tr>
<tr><td rowspan="2">July 29, 2012</td><td colspan="4">M/V Indiana Harbor ballast discharge sampling at the Port of Superior, Wisconsin, in western Lake Superior: TEST CODE: 12-ETV-1D</td></tr>
<tr><td>Tank 5P discharge sampling:<br>02:54 to 04:24</td><td colspan="2">Tank 3P discharge sampling:<br>05:59 to 07:29</td><td>Tank 2P discharge sampling:<br>07:54 to 09:24</td></tr>
<tr><td>July 30, 2012 – August 8, 2012</td><td colspan="4">Data entry, raw data analysis and validation matrix completion</td></tr>
<tr><td>August 8, 2012 – September 7, 2012</td><td colspan="4">Drafting of GSI Interim Technical Report (GSI/SB/QAQC/VR/ETV/1)</td></tr>
<tr><td>September 7, 2012 – September 8, 2012</td><td colspan="4">Verification Organization review and finalization of GSI Interim Technical Report</td></tr>
<tr><td colspan="5"></td></tr>
<tr><td rowspan="11">2</td><td>September 6, 2012 – October 13, 2012</td><td colspan="4">Test/Quality Assurance Plan (TQAP) development, review and finalization</td></tr>
<tr><td rowspan="2">October 17, 2012 – October 18, 2012</td><td colspan="4">M/V Indiana Harbor ballast intake sampling at the Port of Gary, Indiana, in southern Lake Michigan: TEST CODE: 12-ETV-2F</td></tr>
<tr><td colspan="2">Tank 5P intake sampling:<br>21:01 to 22:23</td><td colspan="2">Tank 2P intake sampling:<br>22:59 to 00:06 (18 Oct. 12)</td></tr>
<tr><td>October 18, 2012 – October 21, 2012</td><td colspan="4">M/V Indiana Harbor voyage to Port of Superior, Wisconsin</td></tr>
<tr><td>October 21, 2012</td><td colspan="4">Neutralization of treated ballast tanks 3P and 4P (~13:00 to ~21:30)</td></tr>
<tr><td rowspan="2">October 21, 2012 – October 22, 2012</td><td colspan="4">M/V Indiana Harbor ballast discharge sampling at the Port of Superior, Wisconsin, in western Lake Superior: TEST CODE: 12-ETV-2D</td></tr>
<tr><td>Tank 3P discharge sampling:<br>23:12 to 00:30 (22 Oct. 12)</td><td colspan="2">Tank 4P discharge sampling:<br>01:19 to 02:40</td><td>Tank 5P discharge sampling:<br>04:04 to 05:34</td></tr>
<tr><td>October 23, 2012 – November 9, 2012</td><td colspan="4">Data entry, raw data analysis and validation matrix completion</td></tr>
<tr><td>October 30, 2012 – December 17, 2012</td><td colspan="4">Drafting of GSI Interim Technical Report (GSI/SB/QAQC/VR/ETV/2)</td></tr>
<tr><td>December 18, 2012 – February 1, 2013</td><td colspan="4">Verification Organization review and finalization of GSI Interim Technical Report</td></tr>
</table>

Table 5. Calendar of testing, evaluation, and reporting for Test Cycles 1-4 of *Project 41012* (Continued).

<table>
<tr><th>Test Cycle</th><th>Date</th><th colspan="3">Project Activity</th></tr>
<tr><td rowspan="10">3</td><td>April 17, 2013 – August 2, 2013</td><td colspan="3">Test/Quality Assurance Plan (TQAP) development, review and finalization</td></tr>
<tr><td rowspan="2">August 12, 2013</td><td colspan="3">M/V Indiana Harbor ballast intake sampling at the Port of Muskegon, Michigan, in central Lake Michigan. TEST CODE: 13-ETV-3F</td></tr>
<tr><td colspan="2">Tank 5P intake sampling:<br>18:53 to 20:38</td><td>Tank 2P intake sampling:<br>21:56 to 22:45</td></tr>
<tr><td>August 13, 2013 – August 16, 2013</td><td colspan="3">M/V Indiana Harbor voyage from Port of Muskegon to Port of Duluth-Superior</td></tr>
<tr><td>August 15, 2013</td><td colspan="3">Neutralization of treated ballast tanks 3P and 4P (initiated 18:45)</td></tr>
<tr><td rowspan="2">August 16, 2013</td><td colspan="3">M/V Indiana Harbor ballast discharge sampling at the Port of Superior, Wisconsin, in western Lake Superior: TEST CODE: 13-ETV-3D</td></tr>
<tr><td>Tank 4P discharge sampling:<br>04:14 to 05:22</td><td>Tank 3P discharge sampling:<br>06:56 to 08:25</td><td>Tank 5P discharge sampling:<br>09:45 to 11:15</td></tr>
<tr><td>August 17, 2013 – October 17, 2013</td><td colspan="3">Data entry, raw data analysis and validation matrix completion</td></tr>
<tr><td>October 1, 2013 – October 31, 2013</td><td colspan="3">Drafting of GSI Interim Technical Report (GSI/SB/QAQC/VR/ETV/3)</td></tr>
<tr><td>November 1, 2013</td><td colspan="3">Verification Organization review and finalization of GSI Interim Technical Report</td></tr>
<tr><td rowspan="9">4</td><td>October 26, 2013 – November 5, 2013</td><td colspan="3">Test/Quality Assurance Plan (TQAP) development, review and finalization</td></tr>
<tr><td rowspan="2">November 9, 2013</td><td colspan="3">M/V Indiana Harbor ballast intake sampling at the Port of Ashtabula, Ohio, in southeastern Lake Erie: TEST CODE: 13-ETV-4F</td></tr>
<tr><td>Tank Volume Equivalent #1 intake sampling:<br>03:07 to 04:22</td><td>Tank Volume Equivalent #2 intake sampling:<br>04:22 to 05:09</td><td>Tank Volume Equivalent #3 intake sampling:<br>06:28 to 07:43</td></tr>
<tr><td>November 9, 2013 – November 12, 2013</td><td colspan="3">M/V Indiana Harbor voyage from Port of Ashtabula to Port of Two Harbors</td></tr>
<tr><td rowspan="2">November 12, 2013</td><td colspan="3">M/V Indiana Harbor ballast discharge sampling at the Port of Two Harbors, Minnesota, in western Lake Superior: TEST CODE: 13-ETV-4D</td></tr>
<tr><td>Tank Volume Equivalent #1 discharge sampling:<br>21:30 to 22:44</td><td>Tank Volume Equivalent #2 discharge sampling:<br>22:44 to 23:56</td><td>Tank Volume Equivalent #3 discharge sampling: Aborted due to unexpected change in ship ballast operations</td></tr>
<tr><td>November 13, 2013 – December 3, 2013</td><td colspan="3">Data entry, raw data analysis and validation matrix completion</td></tr>
<tr><td>November 18, 2013 – December 17, 2013</td><td colspan="3">Drafting of GSI Interim Technical Report (GSI/SB/QAQC/VR/ETV/4)</td></tr>
<tr><td>December 17, 2013</td><td colspan="3">Verification Organization review and finalization of GSI Interim Technical Report</td></tr>
</table>

## 2.2 Sample Collection and Analysis Locations

Sample collection and analysis dates and locations for each TC are listed in Table 5, and shown in Figure 2. Intake and discharge ballast sampling always occurred in the ship's engine room. Time-sensitive sample analysis took place in the GSI mobile laboratory (Figure 3) which was located adjacent to the vessel or at a nearby hotel room located 10 – 15 minutes by car from the berthed vessel, depending upon the TC and sample type. Time-sensitive discharge samples were analyzed in laboratories at the GSI Land-Based Research, Development, Testing and Evaluation (RDTE) Facility in Superior, Wisconsin, approximately 15 minutes by car from the docked vessel. Samples having a holding time, specifically those samples for analysis of chemistry parameters and organisms < 10 $\mu$m, collected during both ballast intake and discharge operations were transported per proper sample handling procedures to laboratories of the Lake Superior Research Institute (LSRI) of the University of Wisconsin-Superior in Superior, Wisconsin, for analysis.


Figure 2. Map of the Great Lakes showing Test Cycle 1 - 4 ballast intake and discharge locations.

Figure 3. The GSI mobile laboratory.

## 2.3 Description of the p3SFS Sampling System

All four TCs employed the p3SFS, a skid-mounted sampling system developed by the NRL in Key West, Florida, which the ETV DSP incorporates as an optional sampling approach. Shown in Figure 4, the p3SFS is a closed sampling system containing a flow-through filter device within a compact steel frame that concentrates organisms nominally ≥ 50 $\mu$m entrained in the sample water. NRL personnel installed and commissioned the p3SFS onboard the IH during March and April 2012, with the p3SFS located in the IH's engine room aft of the ballast pumps and directly underneath the ballast header (Figure 5).

In addition, two sample ports were installed in the IH's ballast main at a position where ballast flow was as well-mixed and as fully-developed as practicable, as demonstrated by a computational fluid dynamics (CFD) model simulation of the system. During intake sampling, water was drawn from the ballast stream through one sample port (using an NRL-supplied flange) into the p3SFS, where the samples were collected, then returned to the ballast stream via the second sample port. Following completion of intake sampling, GSI personnel were responsible for switching the hoses connected to the p3SFS inlet and outlet to reverse the sample flow direction for deballasting (see Figure 5 for more details).

The p3SFS was set to automatically maintain a user-selected sample flow rate (up to 11.4 $m^3$/hr) throughout the sampling period. The p3SFS has an input for flow monitoring systems associated with the ballast main flow but did not have a prepared scenario for assuring flow-proportional sampling at the time of this project. Sample water flowed from the intake bent-elbow style sample port (5.1 cm internal diameter, ID) to the p3SFS through a 7.6 m long, 5.1 cm ID rubber hose. The p3SFS filtered the water with two filter housings (FHs) A and B connected in parallel, each containing a removable filter bag constructed of seam-sealed nylon monofilament mesh (35 $\mu$m). The effluent water then passed through a 15.2 m long, 5.1 cm ID rubber hose to the second sample port of similar design and back into the ballast stream. Typically, the p3SFS effluent would be returned downstream of the intake port; however, in the case of the IH installation the return port was upstream of the intake port. The system could nonetheless return at most 11.4 $m^3$/hr, a minimal amount compared to the main ballast flow of approximately 2,200 $m^3$/hr and considered not enough to alter experimental results.

Sensors within the p3SFS were set to measure key operational parameters (i.e., sample flow, main ballast flow signal input, temperature, turbidity, inlet and outlet pressure, differential pressure, etc.), and resulting data were recorded by a data logger. The p3SFS provided temperature and turbidity data, measured via the system's in-line sensors, in two formats: continuous in-line data automatically recorded every second and accessible electronically as a Microsoft Excel file; and a summary of the continuous in-line data displayed on the p3SFS at the conclusion of each sampling event. The latter data was recorded by hand.

The p3SFS's drip sampler, located immediately upstream of the FHs, captured flow-controlled whole water samples. This integrated whole water sample was then divided and used for enumeration of organisms ≥ 10 $\mu$m and < 50 $\mu$m, organisms < 10 $\mu$m and analysis of water quality parameters, as well as disinfection byproducts and WET testing if applicable.

GSI use of the p3SFS was consistent with p3SFS installation and operation guidelines (NRL, 2012), and p3SFS developer e-mails to GSI personnel regarding p3SFS sample collection. Consistent with these guidelines, GSI sampling using the p3SFS was terminated prior to collection of the entire tank ballasting/deballasting period if:

- The p3SFS sensors around the filter bags indicated a pressure differential equal to or greater than 5 psi (0.3 bar);
- The ballasting operation ceased;
- 6 $m^3$ of sample was concentrated; or
- More than 90 minutes elapsed.

The p3SFS unit was the same for the four TCs, except prior to TC3, the turbidity sensor on the p3SFS was replaced and the p3SFS's software was updated (9 August 2013). In addition, prior to TC4, NRL upgraded the p3SFS to enable sequential use of the two filter canisters to allow continuous sample flow under high TSS conditions. This alteration permitted a sequential pattern of sampling with canister A and B, allowing technicians to recommission individual canisters upon clogging without interrupting sample collection or ballasting processes.

GSI received seven filter bags with the p3SFS, and designated three for untreated samples and four for treated samples. This separation ensured no contamination of live organisms from intake samples into discharge samples. The p3SFS sample collection procedure also involved thoroughly rinsing of filter bags to ensure collection of all concentrated organisms. Therefore, after concentrate collection from each FH, filter bags were well rinsed and ready for sample collection use during the next experimental ballast tank.

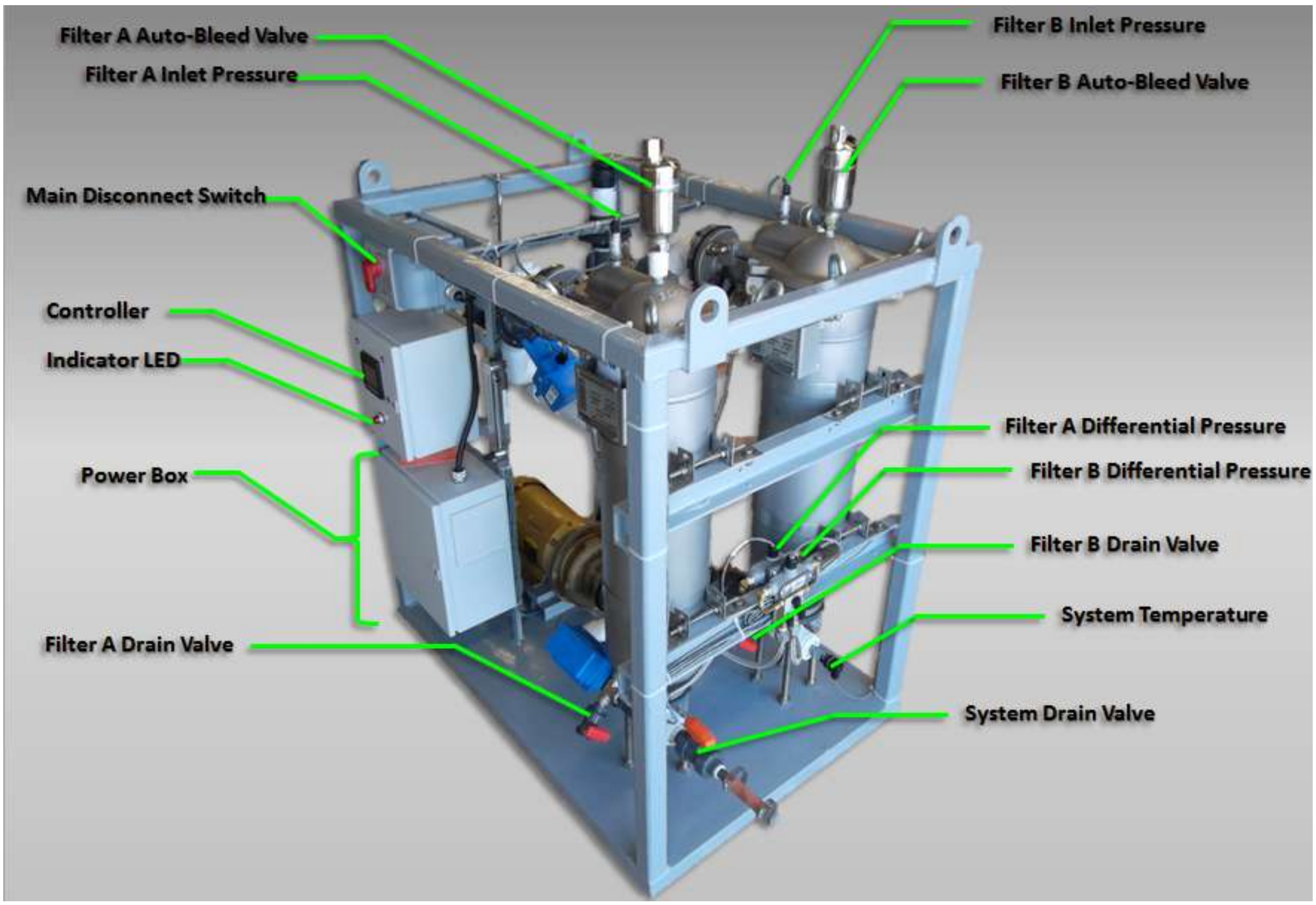


Figure 4. Side view of the Prototype Three Skid Filter System (p3SFS).

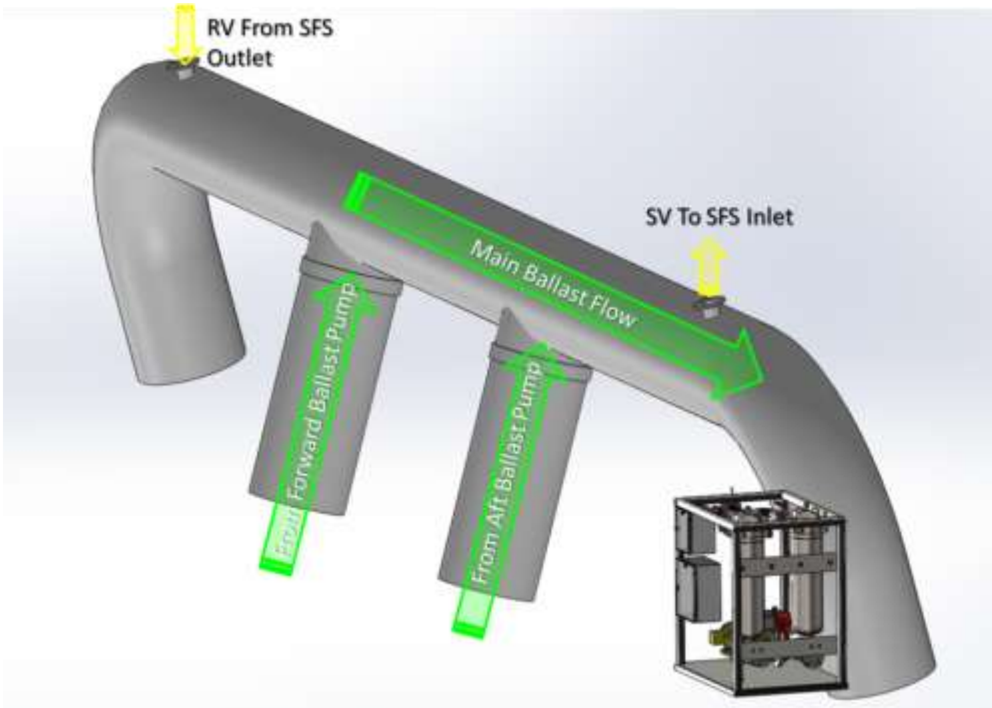


Figure 5. Three-dimensional drawing of the Prototype Three Skid Filter (p3SFS) showing placement in the M/V *Indiana Harbor*'s engine room with respect to the ballast header during discharge. Note: during intake the flow in the header travels in the opposite direction to the ballast tanks and the SV and RV are switched for sample collection.

## 2.4 Sample Collection

Tables 6 and 7 list the operational data, and water quality/chemistry, biological and external collaborator samples collected during TCs 1-4 ballast intake and discharge operations. Overall, intake sample collection methods were similar throughout TCs 1-4, with the exception of tank ballasting and deballasting order (Table 5).

Consistent with each TC's TQAP (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b), three or more GSI team members boarded the IH once it had docked at the respective intake or discharge location (Figures 2 and 6). Supplies were immediately loaded onboard the vessel and personnel set up for sample collection in the engine room. The GSI Engineer initiated sampling using the prompt on the p3SFS's human machine interface (HMI). He recorded the start time in a bound laboratory notebook that was uniquely-identified by coding and specific to *Project 41012*. The GSI Test Manager also recorded the start time on a pre-printed datasheet during TCs 1-3.

During sampling the GSI Engineer observed FHs A and B inlet and outlet pressures to monitor if the pressure differential was increasing to a level that would require switching the nets, i.e., ≥ 5 psi. He also monitored the drip sampler throughout sampling and adjusted the flow rate if outside the target value. As required, the GSI Engineer isolated the p3SFS from the IH for sample collection or maintenance.

The GSI Test Manager conducted sample collection and processing. Immediately upon completion of a sampling interval, the GSI Test Manager isolated the FHs by closing the inlet and outlet valves. He then

drained the filtrate water from the bottom drain on each of the FHs prior to unsealing the FH lid. Next he added the filtrate water (each filter canister held 27.4 L) to a manual pump sprayer and sprayed each filter bag (Figure 7) from the top of the filtrate bag towards the bottom to rinse the organisms off the filter bag (Figure 8). The samples were then concentrated to 1 L for subsequent analysis. In addition, the GSI Test Manager reserved 2 L of filtrate water per canister for use in processing the samples.

Figure 6. GSI sample collection team waiting to board the M/V *Indiana Harbor*.


Figure 7. Full p3SFS filter bag after completion of a sampling event.

Figure 8. GSI sample collection team rinsing the inside of the p3SFS filter bag.

An additional GSI staff person collected time-integrated whole water samples from the p3SFS drip sampler. After agitating the carboys to mix the sample water, GSI personnel collected subsamples for analysis of organisms ≥ 10 $\mu$m and < 50 $\mu$m and organisms < 10 $\mu$m. The remaining carboy contents were mixed again and subsampled for analysis of TSS, POM, %T, NPOC and DOC.

No water chemistry samples were collected during TC4 discharge because sampling and analysis were abbreviated during this TC. No samples were collected for analysis of organisms ≥10 $\mu$m and <50 $\mu$m during TC4 discharge, and no samples were collected for analysis of organisms < 10 $\mu$m during TC4 intake or discharge. During the TC 2 and 3 discharge evolutions, a subsample also was collected for analysis of disinfection byproducts and WET. The remaining sample water was used to measure temperature, dissolved oxygen, pH, turbidity, salinity, specific conductivity and total chlorophyll; for QA/QC purposes; and in one case, for external collaborators.

Following sample collection, GSI personnel transferred samples off the ship to analysts in accordance with GSI chain of custody (COC) procedures. Following completion of all intake and discharge sampling activities, the GSI Engineer and GSI Test Manager remained on board the IH to restore the engine room to its pre-sampling condition.

### 2.4.1 Determination of Proportionality of Sample Flow to Ballast Flow

GSI team members recorded the tank height of each tank being ballasted/deballasted using the ballast tank level display in the IH Control Room (Figure 9). Tank heights were recorded on a pre-printed datasheet at various time points throughout each sampling operation. Following each sampling event, the heights were entered into a Microsoft Excel spreadsheet and sent to the GSI Engineer who converted tank heights to volumes using ballast tank diagrams provided by the IH Chief Engineer. The volume of water ballasted/deballasted from each tank or TVE was compared to the volume of water sampled by the p3SFS to indirectly determine sample flow to ballast flow proportionality.

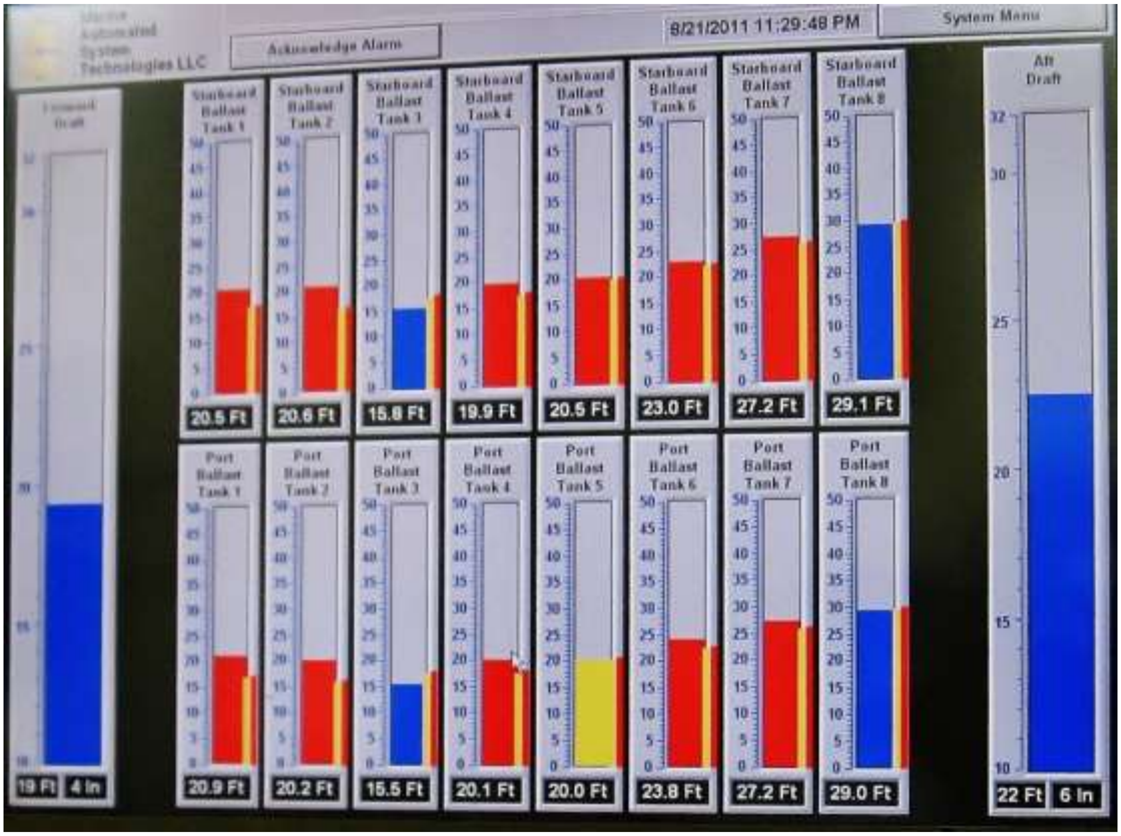


Figure 9. Ballast tank levels displayed in the M/V *Indiana Harbor* control room.

## 2.5 Sample Handling and Storage

Sample handling and storage requirements, including holding conditions and specific preservatives, for samples collected during TCs 1-4 intake and discharge operations are detailed in Table 8. The GSI Senior QAQC Officer assigned unique sample codes to each type of sample as described in the TC-specific TQAPs (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b).

Table 6. Class, type and number of samples collected during Test Cycle 1-4 ballast intake operations.

| Parameter Category | Parameter | Measurement Class | Sample Type | Number of Replicate Samples Collected per Tank | Sample Volume per Replicate |
|---|---|---|---|---|---|
| Operational | p3SFS volume sampled | Core | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| | Ballast system flow rate | Core | Discrete | Tank height recorded < every 10 minutes, i.e., ≥ 5 readings | N/A - Measurement |
| | p3SFS flow rate | Core | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| | p3SFS differential pressure | Core | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| | Drip sample volume | Core | Discrete | N/A - Measurement | N/A - Measurement |
| | Drip sample flow rate | Core | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| Not Applicable – External Collaboration (TC 3 only) | Environmental eDNA research and development | Auxiliary | Time integrated from 19 L carboy | 1 only from tank 5P | 900 to 1000 mL |
| Water Chemistry | Temperature | Core | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| | Turbidity | Auxiliary | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| | Temperature, dissolved oxygen/percent saturation, pH, turbidity, salinity, specific conductivity and total chlorophyll | Core | Time integrated from 19 L carboy | 1 | 600 to 1000 mL |
| | Total suspended solids, particulate organic matter and percent transmittance | Core | Time integrated from 19 L carboy (TCs 1 – 3); grab samples off main line (TC4 intake) | 3 | 900 to 1000 mL |
| | Total organic carbon as non-Purgeable organic carbon and dissolved organic matter as dissolved organic carbon | Core | Time integrated from 19 L carboy; grab samples off main line (TC4 intake) | 3 | 100 to 125 mL |
| Biology | Organisms ≥ 50 $\mu$m | Core | Time integrated from p3SFS | 1 - 2 | ~ 6 $m^3$ ± 10 % |
| | Organisms ≥ 10 and < 50 $\mu$m (TCs 1 – 3 only) | Core | Time integrated from 19 L carboy | 1 | 900 to 1000 mL |
| | Organisms < 10 $\mu$m: total heterotrophic bacteria, total coliform bacteria, *E. coli*, and *Enterococcus spp.* (TCs 1 – 3 only) | Core | Time integrated from 19 L carboy | 3 | 900 to 1000 mL |
| | | Core (matrix blank) | Time integrated from 19 L carboy | 1 | 1900 to 2000 mL |

Table 7. Class, type and number of samples collected during Test Cycle 1-4 ballast discharge operations.

| Parameter Category | Parameter | Measurement Class | Sample Type | Number of Replicate Samples Collected per Tank | Sample Volume per Replicate |
|---|---|---|---|---|---|
| Operational | p3SFS volume sampled | Core | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| | Ballast system flow rate | Core | Discrete | Tank height recorded < every 10 minutes, i.e., ≥ 5 readings | N/A - Measurement |
| | p3SFS flow rate | Core | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| | p3SFS differential pressure | Core | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| | Drip sample volume | Core | Discrete | N/A - Measurement | N/A - Measurement |
| | Drip sample flow rate | Core | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| Not Applicable – External Collaboration (TC3 only) | Environmental eDNA research and development | Auxiliary | Time integrated from 50 L carboy | 1 (5P only) | 1900 to 2000 mL |
| | Variable fluorometer prototype methods development | Auxiliary | Time integrated from 50 L carboy | 1 (4P and 5P only) | 500 to 1000 mL |
| Water Chemistry | Temperature | Core | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| | Turbidity | Auxiliary | *In situ*, continuous | N/A - Measurement | N/A - Measurement |
| | Temperature, dissolved oxygen/percent saturation, pH, turbidity, salinity, specific conductivity and total chlorophyll | Core | Time integrated from 19 L carboy (TCs 1 and 4); time integrated from 50 L carboy (TCs 2 and 3) | 1 | 600 to 1000 mL |
| | Total suspended solids, particulate organic matter and percent transmittance | Core | Time integrated from 19 L carboy (TC1); time integrated from 50 L carboy (TCs 2 and 3); no samples collected for TC4 | 3 | 900 to 1000 mL |
| | Total organic carbon as non-purgeable organic carbon and dissolved organic matter as dissolved organic carbon | Core | Time integrated from 19 L carboy (TC1); time integrated from 50 L carboy (TCs 2 and 3); no samples collected for TC4 | 3 | 100 to 125 mL |
| | Disinfection byproducts (trihalomethanes, haloacetic acids, chlorate, bromate and sodium) | Core | Time integrated from 50 L carboy (TCs 2 and 3 only) | 1 | 1 L (divided into containers provided by analytical laboratory) |

Table 7. Class, type and number of samples collected during Test Cycle 1-4 ballast discharge operations (Continued).

| Parameter Category | Parameter | Measurement Class | Sample Type | Number of Replicate Samples Collected per Tank | Sample Volume per Replicate |
|---|---|---|---|---|---|
| Whole Effluent Toxicity (WET) | Whole effluent toxicity | Auxiliary | Time integrated from 50 L carboy (TCs 2 and 3 only) | 1 | 32 – 36 L for treated discharge; 13 to 19 L for untreated discharge |
| Biology | Organisms ≥ 50 $\mu$m | Core | Time integrated from p3SFS | 1-2 | 4.57 – 6.02 $m^3$ |
| | Organisms ≥ 10 and < 50 $\mu$m | Core | Time integrated from 19 L carboy (TC1); time integrated from 50 L carboy (TCs 2 and 3). No samples collected for TC4 | 1 | 900 to 1000 mL |
| | Organisms < 10 $\mu$m: total heterotrophic bacteria, total coliform bacteria, *E. coli*, and *Enterococcus* spp. | Core | Time integrated from 19 L carboy (TC1); time integrated from 50 L carboy (TCs 2 and 3). No samples collected for TC4 | 3 | 900 to 1000 mL |
| | | Core (matrix blank) | Time integrated from 19 L carboy (TC1); time integrated from 50 L carboy (TCs 2 and 3). No samples collected for TC4 | 1 | 1900 to 2000 mL |

Table 8. Sample handling and storage requirements of samples collected during Test Cycle 1-4.

| Parameter | Container | Sample Volume | Processing/Preservation | Maximum Holding Time |
|---|---|---|---|---|
| Electronic Continuous, In-Line Operational Data (Volume, Ballast System and p3SFS Flow Rate, Differential Pressure) | N/A - Measurement | N/A - Measurement | Digital archive maintained. | N/A - Measurement |
| Electronic Continuous, In-Line Data (Temperature and Turbidity) | N/A - Measurement | N/A - Measurement | Digital archive maintained. | N/A - Measurement |
| Total Suspended Solids, Particulate Organic Matter and Percent Transmittance | 1 L HDPE | 900 to 1000 mL | Analyzed immediately; or refrigerated. | 24 hours |
| Total Organic Carbon as Non-Purgeable Organic Carbon | 125 mL Borosilicate glass | 100 to 125 mL | HCl added to pH < 2. Analyzed immediately or refrigerated. | 28 days |
| Dissolved Organic Matter as Dissolved Organic Carbon | 125 mL Borosilicate glass | 100 to 125 mL | Filtered, HCl added to pH < 2. Analyzed immediately or refrigerated. | 28 days |
| Disinfection Byproducts (i.e., Trihalomethanes, Haloacetic Acids, Chlorate, Bromate and Sodium) | 1 L HDPE | 900 to 1000 mL | Specific to TCs 2 and 3 discharge samples only. Samples were transferred to appropriate sample bottles and shipped overnight in a cooler packed with ice as per Analytical Laboratory Services' instructions for collection/preservation. | Trihalomethanes and haloacetic acid: 14 days. Sodium: 6 months. Bromate: 28 days. Chlorate: 28 days. |
| Whole Effluent Toxicity (WET) | Transfer into 19 L HDPE carboy | 32 - 36 L for treated discharge and 13 to 19 L for untreated discharge | Specific to TCs 2 and 3 discharge only. Placed on ice in large coolers and transported to laboratory. Refrigerated if immediate analysis was not possible. | Test set up within 24 hours of sample receipt. Whole effluent held for the duration of the WET Testing (up to 8 days). Prepared dilution water (DSH water) holding time was 11 days. |

Table 8. Sample handling and storage requirements of samples collected during Test Cycle 1-4 (Continued).

| Parameter | Container | Sample Volume | Processing/Preservation | Maximum Holding Time |
|---|---|---|---|---|
| Organisms ≥ 50 $\mu$m | 1 L cod end | 4.57 – 6.02 $m^3$ to 1 L | Live samples processed and analyzed within 3.5 hours of collection. Unanalyzed samples preserved using formalin solution. | Maximum hold time of 6 hours from collection. Samples that were preserved in lieu of live/dead analysis were preserved immediately. |
| Organisms ≥ 10 and < 50 $\mu$m | 1 L HDPE | 900 to 1000 mL | Stained with Fluorescein Diacetate (FDA). Processed and analyzed within 1.5 hours of collection. Unanalyzed samples preserved using Lugol's solution. Alternatively, for TC3 and TC4 intake, samples were preserved with 10 mL of Lugol's solution and analyzed with 72 hours of collection. | Maximum hold time of 2 hours from collection. Samples that were preserved in lieu of live/dead analysis were preserved immediately. |
| Organisms < 10 $\mu$m: Total Heterotrophic Bacteria, Total Coliform Bacteria, *E. coli*, and *Enterococcus spp.* | 1 L sterile PP | 900 to 950 mL (leave 2.5 cm of headspace in the bottle) | Placed on ice in coolers and transported to laboratory for immediate analysis. Refrigerated if immediate analysis was not possible. Note: no microbial samples were collected on intake or discharge for TC4. | 24 hours |

## 2.6 Sample Analysis

### 2.6.1 Water Chemistry Detection Limits

Prior to the start of the 2012 and 2013 GSI shipboard testing seasons, the method detection limit (MDL) and limit of quantification (LOQ) for the water chemistry parameters that GSI directly measured in TCs 1 through 4 (i.e., TSS, NPOC, DOC and POM) were determined following relevant GSI and LSRI standard operating procedures (SOPs). The 2012 and 2013 MDLs and LOQs for TSS, NPOC, DOC and POM are listed in Table 9.

Table 9. 2012 and 2013 GSI method detection limits and limit of quantifications for total suspended solids, non-purgeable organic carbon, dissolved organic carbon and particulate organic matter.

| Year | Parameter | Determination Date | Method Detection Limit (MDL) | Limit of Quantification (LOQ) |
|---|---|---|---|---|
| 2012 | Total Suspended Solids | 23 May 2012 | 1.07 mg/L | 3.57 mg/L |
| | Non-Purgeable Organic Carbon | 28 June 2012 | 0.11 mg/L | 0.35 mg/L |
| | Dissolved Organic Carbon | 28 June 2012 | 0.11 mg/L | 0.35 mg/L |
| | Particulate Organic Matter | 25 September 2012 | 0.45 mg/L | 1.50 mg/L |
| 2013 | Total Suspended Solids | June 4 2013 | 0.78 mg/L | 2.60 mg/L |
| | Non-Purgeable Organic Carbon | May 29 2013 | 0.20 mg/L | 0.65 mg/L |
| | Dissolved Organic Carbon | May 29 2013 | 0.20 mg/L | 0.65 mg/L |
| | Particulate Organic Matter | June 4 2013 | 0.59 mg/L | 1.96 mg/L |

### 2.6.2 Total Suspended Solids, Particulate Organic Matter, Percent Transmittance and Mineral Matter

Intake and discharge samples collected for analysis of TSS, POM (TCs 2-4), %T and mineral matter (MM) during TCs 1-4 are listed in Tables 6 and 7, respectively. These samples were analyzed according to the TC-specific TQAPs (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b). POM is a filter, dry and combust method. The ETV DSP assumes that the POM concentration is generally about twice the POC concentration.

### 2.6.3 Non-Purgeable Organic Carbon, Dissolved Organic Carbon and Particulate Organic Carbon

Intake and discharge samples collected for analysis of NPOC, DOC and POC (POC is the difference between measured NPOC and DOC) during TCs 1-4 are listed in Tables 6 and 7, respectively. These samples were analyzed according to the TC-specific TQAPs (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b).

### 2.6.4 YSI Multiparameter Water Quality Sonde Measurements

Water quality parameters measured during TC1-4 using a YSI Multiparameter Water Quality Sonde are listed in Tables 6 and 7. These measurements were analyzed according to the TC-specific TQAPs (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b).

### 2.6.5 Biology

Intake and discharge samples collected during TCs 1-4 for biological analysis are listed in Tables 6 and 7, respectively. These samples were analyzed according to procedures detailed the TC-specific TQAPs (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b; Figure 10).

Figure 10. GSI personnel conducting analysis of organisms ≥ 10 $\mu$m and < 50 $\mu$m.

#### 2.6.6 Disinfection Byproducts

Intake and discharge samples collected for analysis of selected disinfection byproducts during TC2 and TC3 (i.e., samples were not collected during TC1 and TC4 due to the lack of a BWMS) are listed in Tables 6 and 7, respectively. These samples were analyzed according to the TC-specific TQAPs (GSI, 2012b; GSI, 2013a).

#### 2.6.7 Whole Effluent Toxicity

Discharge samples collected for analysis of WET during TCs 2 and 3, in which the BWMS was active, are listed in Tables 6 and 7, respectively. These samples were analyzed according to procedures detailed in the TC-specific TQAPs (GSI, 2012b; GSI, 2013a).

### 2.7 Data Processing, Verification, Validation and Storage

GSI personnel recorded sample collection and operational data according to procedures detailed in the TC-specific TQAPs (GSI, 2012a; GSI, 2012b; GSI, 2013a; GSI, 2013b). Completed data collection forms were secured in uniquely-identified three ring binders specific to *Project 41012*. Biological and chemical data that were recorded by hand were manually entered into either a Microsoft Access Database or a Microsoft Excel Spreadsheet.

A percentage of data that was recorded by hand and entered into Microsoft Access or Excel was verified against the original raw data by the GSI Senior QAQC Officer. This procedure also included verification of the accuracy of computer-generated data through hand-calculation. The percentage of verified raw data depended upon the amount of raw data that was generated, and ranged from 10 % to 100 % of the original raw data.

All electronic data files are stored on the LSRI's secured Local Area Network that can be accessed only by relevant GSI personnel. The electronic data files are also stored on the GSI's internal SharePoint website (greatshipsinitiative.net), which acts as a secondary data backup/storage mechanism. In addition, the GSI Senior QAQC Officer is responsible for archiving and storing all original raw data applicable to *Project 41012* in a climate-controlled, secure archive room at the LSRI for a period seven years following finalization of this document.

# 3 BALLAST WATER MANAGEMENT SYSTEM PERFORMANCE RESULTS

This section presents information relevant to BWMS evaluation using the ETV DSP. Because the prototype BWMS was operative in TC2 and TC3 only, operational, biological and environmental performance results sections reflect information derived from only those two test cycles.

Results describe:

- BWMS operational outcomes (TC2 and TC3, only);
- Sampling operations;
- Characterization of ballast water sampled in TCs 1-4;
- Characterization and assessment of challenge conditions;
- Biological performance of the prototype NaOH BWMS (TC2 and TC3, only);
- Environmental acceptability of the prototype NaOH BWMS (TC2 and TC3, only); and
- QA/QC, including data quality indicators and TQAP deviations.

## 3.1 Ballast Water Management System Operational Outcomes

The prototype NaOH BWMS operated (TC2 and TC3, only) consistently with BWMS developer objectives.

### 3.1.1 Test Cycle 2

According to the BWMS Developer, ballast treatment during TC2 took place as planned. The measured pH of the water in the two ballast tanks after treatment with the prototype NaOH BWMS was not provided to GSI. The IH retained the treated water onboard for the desired retention time (approximately 3 days), and the water achieved the desired pH of 6.0 – 8.8 after neutralization. The BWMS Developer also confirmed successful neutralization prior to ballast discharge on 12 October 2012 by completing and signing *GSI FORM: Ballast Water Management System Neutralization Verification*.

### 3.1.2 Test Cycle 3

The BWMS Developer reported that the prototype NaOH BWMS operated as planned during TC3, with a few deviations summarized here:

- Approximately 5 cm of clay-like material covered the bottom of both treatment ballast tanks, with the material up to 12 cm deep in certain areas (mainly isolated to a few sections under the ballast line) despite cleaning of both tanks prior to TC3;
- The prototype NaOH BWMS achieved a pH of 11.7 in tanks 3P and 4P, rather than the expected pH of 12.0;
- Tank 3P took longer to neutralize than tank 4P due to a slower flow rate of $CO_2$; and

- The IH's crew had to introduce raw water into the vessel's ballast pump seals towards the end of one of the treatment tank discharges and a small amount of contaminated water was introduced.

The IH retained the treated water for the desired retention time of approximately three days, and the water in the two treated tanks achieved the desired pH of 6.0 – 8.8 after neutralization. The BWMS developer confirmed successful neutralization prior to ballast discharge on 16 August 2013 by completing and signing *GSI FORM: Ballast Water Management System Neutralization Verification*. Prior to ballast discharge the GSI Test Manager also verified that complete neutralization of the NaOH-treated ballast water had occurred.

## 3.2 Sampling Operations

### 3.2.1 Intake Sampling

Table 10 summarizes p3SFS operating conditions during TC 1-4 intake sampling events.

#### *3.2.1.1 Test Cycle 1*

TC1 target operational conditions were met without interruption during intake of tanks 5P and 3P (Table 10). Approximately 50 minutes into tank 2P intake operation, the ballast pump on the IH was stopped for 2 minutes due to cargo loading operations, and the p3SFS sampling operation was paused. After the pause, the IH's ballast pump and the p3SFS were restarted, however, the p3SFS automatic pump start-up failed. Although the p3SFS display read "Sampling" approximately 18 minutes after the restart, GSI personnel observed that the p3SFS display was reading "0 US GPM." Consequently, approximately 21 minutes of tank 2P's 90 minute intake operation was not sampled, which was approximately 23 % of the total sampling operation time. As a result, less water, i.e., 4.7 $m^3$[1], was filtered during tank 2P's intake sampling operation, which fell outside the target range of 6.0 $m^3$ ± 10 %[2] (Table 10).

The operational data summary of the sampling operation during TC1 tank 2P intake also displayed lower values than the auto-logged electronic data, with only the latter falling within the target range (Table 10). Moreover, several discrepancies were noted with the automatically-logged data. For example, the p3SFS is programmed to measure and record operational data once every second, such that the 69 minute intake operation for tank 2P should have yielded 4,140 data points, rather than the 3,747 data points obtained (approximately 10 % lower than expected) (Table 10). Similarly, the 94 minute intake operation for tank 5P produced 4,899 data points, about 13 % less than expected, and the 91 minute intake operation for tank 3P had 5,034 data points, about 8 % less than expected (Table 10).

#### *3.2.1.2 Test Cycle 2*

TC2 sampling was paused to accommodate IH cargo operations during intake of tanks 5P and 2P; data provided in Table 10 are therefore either an average of the pre- and post-pause data, or the sum of the two parts, depending on the specific parameter (Table 10). Based on historical data, GSI anticipated tank 2P and 5P ballasting times to be 70 to 100 minutes, which would accommodate the planned sampling duration of 90 minutes. However, ballasting times were 61 minutes for tank 5P and only 49 minutes for tank 2P. This high ballasting rate truncated sampling time, causing GSI to miss several operational targets, including volume

[1] Based on assumption that p3SFS flow meter was accurately recording flow rates.
[2] As above.

sampled, drip sample volume and drip sample flow rate (Table 10). The average sample flow rate, average pressure and average differential pressure for tank 2P could not be calculated because the pre-pause data from the first 29 minutes of sampling was lost when the p3SFS's sampling condition was inadvertently changed to "Stop" rather than "Pause". Instead, averages calculated from the post-pause sampling data of 20 minutes are provided in Table 10, and are well inside the target range for these specific parameters[3].

#### *3.2.1.3 Test Cycle 3*

During tank 2P intake, sampling was stopped 49 minutes into the ballast operation due to the pressure differential of the p3SFS reaching 5 psi. However, with only 10 minutes of the IH's scheduled ballast operation remaining, the GSI PI made a decision not to resume sampling. Similarly, sampling of tank 5P was paused for 26 minutes part-way through the intake operation when the pressure differential rose above 5 psi[4]. The clogged filters were replaced during this pause, and sampling resumed. Truncated sampling operations during TC3 and, like for TC2, resulted in several operational targets not being met, including volume sampled[5], drip sample volume and drip sample flow rate (Table 10).

#### *3.2.1.4 Test Cycle 4*

In TC4, several operational parameters missed their target ranges due to circumstances outside GSI's control (Table 10). For example, during TVE#2, GSI ceased sampling operations at 37 minutes owing to an anticipated prolonged pause in ballasting. As a result, tank heights were recorded at only two time points (at the beginning and near the end of the sampling operation) and the average main ballast flow could not be adequately calculated from the number of tank heights recorded (Table 10; Figure 9). The main ballast flow rate for TVE#1 was also below the recommended range for subisokinetic sampling (i.e., ≥ 1700 $m^3$/Hr) (Table 10). The flow rate through the p3SFS drip sampler was also significantly slower than planned (Table 10). In addition, GSI personnel detected a crack in the sampler's plastic nipple that produced sample water leakage just upstream of the drip sampler shut off valve. This leak reduced drip sample volumes to below the target range for both TVE#1 and TVE#2 (Table 10).

[3] Based on assumption that p3SFS flow meter was accurately recording flow rates.
[4] As above.
[5] As above.

Table 10. Summary of p3SFS operating conditions during *Project 41012* intake sampling events. For Test Cycle 1, output includes hand recorded and auto-logged electronic data. The auto-logged data is provided in parenthesis. For all TCs, values marked with an asterisk (*) are outside the valid range for that parameter.

| Test Cycle | Parameter | Valid Range | Tank 5P | Tank 2P | Tank 3P |
|---|---|---|---|---|---|
| 1 | Sampling duration (min) | ≤ 90 | 94 | 69 | 91 |
| | Volume sampled ($m^3$) | 6 ± 10 % | 6.0 | 4.7* | 6.0 |
| | Ballast flow rate ($m^3$/Hr) | ≥ 1700 | Did not determine | Did not determine | Did not determine |
| | Sample flow rate ($m^3$/hour) | 4 ± 10 % | 4.0 (3.9, *n*=4899) | 0.8* (3.8, *n*=3747) | 4.0 (3.9, *n*=5034) |
| | Pressure housing A (bar) | No requirement | 1.68 (1.67, *n*=4899) | 0.46 (1.63, *n*=3747) | 1.52 (1.52, *n*=5034) |
| | Pressure differential housing A (bar) | < 0.3 | - 0.01 (- 0.005, *n*=4899) | 0.00 (0.01, *n*=3747) | 0.02 (0.02, *n*=5034) |
| | Pressure housing B (bar) | No requirement | 1.73 (1.73, *n*=4899) | 0.47 (1.69, *n*=3747) | 1.58 (1.58, *n*=5034) |
| | Pressure differential housing B (bar) | < 0.3 | - 0.07 (- 0.07, *n*=4899) | - 0.01 (- 0.06, *n*=3747) | - 0.05 (- 0.04, *n*=5034) |
| | Drip sample volume (L) | No requirement | 11 | 13 | 12 |
| | Drip sample flow rate (L/Hr) | No requirement | 7.0 | 11.3 | 7.9 |
| Test Cycle | Parameter | Valid Range | Tank 5P | Tank 2P | |
| 2 | Sampling duration (min) | ≤ 90 | 61 | 49 | |
| | Volume sampled ($m^3$) | 5.4 – 6.6 | 4.2* | ~3.4* | |
| | Ballast flow rate ($m^3$/Hr) | > 2,000 | 3490 | 3224 | |
| | Sample flow rate ($m^3$/hour) | 3.6 – 4.4 | 4.0 | 4.1 (Post-Pause data only) | |
| | Pressure housing A (bar) | No requirement | 1.98 | 2.01 (Post-Pause data only) | |
| | Pressure differential housing A (bar) | < 0.3 | 0.04 | 0.03 (Post-Pause data only) | |
| | Pressure housing B (bar) | No requirement | 2.15 | 2.19 (Post-Pause data only) | |
| | Pressure differential housing B (bar) | < 0.3 | 0.10 | 0.08 (Post-Pause data only) | |
| | Drip sample volume (L) | 13.5 – 16.5 | 13* | 10* | |
| | Drip sample flow rate (L/Hr) | 9 - 11 | 12.8* | 12.2* | |
| Test Cycle | Parameter | Valid Range | Tank 5P | Tank 2P | |
| 3 | Sampling duration (min) | ≤ 90 | 79 | 49 | |
| | Volume sampled ($m^3$) | 1.6 – 10.4 (Target = 6) | 5.16 | 3.33 | |
| | Ballast flow rate ($m^3$/Hr) | ≥ 1,700 | 2,584 | 2,054 | |
| | Sample flow rate ($m^3$/hour) | 1 – 7 (Target = 4) | 4.12 | 3.92 | |
| | Pressure differential housing A (psi) | ≤ 5 | ≤ 5.40* | ≤ 5.48* | |
| | Pressure differential housing B (psi) | ≤ 5 | ≤ 5.38* | ≤ 5.63* | |
| | Drip sample volume (L) | 10 – 19 (Target = 15) | 11.5 | 8.0* | |
| | Drip sample flow rate (L/Hr) | 7 – 13 (Target = 10) | 8.7 | 9.8 | |

Table 10. Summary of p3SFS operating conditions during *Project 41012* intake sampling events. For Test Cycle 1, output includes hand recorded and auto-logged electronic data. The auto-logged data is provided in parenthesis. For all TCs, values marked with an asterisk (*) are outside the valid range for that parameter (Continued).

| Test Cycle | Parameter | Valid Range | Tank 5P | Tank 2P | Tank 3P |
|---|---|---|---|---|---|
| Test Cycle | Parameter | Valid Range | Tank Volume Equivalent #1 | Tank Volume Equivalent #2 | Tank Volume Equivalent #3 |
| 4 | Sampling duration (min) | 75 | 75 | 37* | 75 |
| | Volume sampled ($m^3$) | 1.6 – 10.4 (Target = 5) | 5.1 | 2.6 | 5.1 |
| | Ballast flow rate ($m^3$/Hr) | ≥ 1,700 | 1,322* | Could not determine; too few tank heights recorded. | 2,142 |
| | Sample flow rate ($m^3$/hour) | 1 – 7 (Target = 4) | 4.1 | 4.2 | 4.1 |
| | Average differential pressure (psi) | ≤ 5 | 1.2 | 2.1 | 0.9 |
| | Drip sample volume (L) | 10 – 19 (Target = 15) | 8* | 4* | 10 |
| | Drip sample flow rate (L/Hr) | 7 – 13 (Target = 10) | 6.4* | 6.5* | 8.0* |

### 3.2.2 Discharge Sampling

Table 11 summarizes p3SFS operating conditions during the discharge sampling events.

#### *3.2.2.1 Test Cycle 1*

For TC1 discharge, all target operational conditions were met (Table 11). However, Table 11 displays only hand-recorded operational data; the Micro Secure Digital (SD) Card used to save the continuously collected electronic data was not properly formatted by GSI prior to data collection.

#### *3.2.2.2 Test Cycle 2*

Sample collection for the three tanks was uninterrupted during the TC2 discharge operation. The sample collection time was, however, shortened when the ship's crew decided to discharge less water than expected resulting in the target value for total volume sampled from tank 3P likely not being met[6] (Table 11). The flow rate of the drip sampler during tank 3P discharge was increased to 35 L/hour to compensate for the reduced ballasting time to ensure that an acceptable volume of whole water was collected (42 L; Table 11). As a result, the drip sampler flow rate was above the valid range of 27 – 33 L/hour (Table 11). Similarly, tank 3P sample volume fell slightly below the target volume range[7] (Table 11).

#### *3.2.2.3 Test Cycle 3*

GSI increased the drip sample flow rate for Tank 4P above the target prior to the start of tank discharge to ensure an adequate sample volume was available for WET testing due to an expected abbreviated ballast pumping duration (Table 11).

#### *3.2.2.4 Test Cycle 4*

The target operating conditions were met for the two TVEs sampled during the TC4 discharge. The third TVE was not collected because IH deballasting operations were paused for a prolonged period. The average main ballast flow for TVE#2 on discharge could not be calculated because an insufficient number of tank heights were recorded (Table 11). An equipment malfunction also reduced the drip sample flow rates for TVE#1 and TVE#2 below the target range[8] (Table 11; Figure 11). This malfunction also reduced drip sample volumes below the valid range (Table 11).

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[6] Based on assumption that p3SFS flow meter was accurately recording flow rates.
[7] As above.
[8] On intake the GSI sample team detected a crack leaking water in the plastic nipple located just prior to the p3SFS drip sampler shut off valve. GSI assumed the leak was causing the slow drip sampler flow rate, and attempted repair with negative results.


Figure 11. Cracked nipple on the p3SFS leading to the drip sampler.

Table 11. Summary of p3SFS operating conditions during Project 41012 discharge sampling events. Values marked with an asterisk (*) are outside the valid range for that parameter.

| Test Cycle | Parameter | Valid Range | Tank 5P | Tank 2P | Tank 3P |
|---|---|---|---|---|---|
| 1 | Sampling duration (min) | ≤ 90 | 90 | 90 | 90 |
| | Volume sampled ($m^3$)[9] | 6 ± 10 % | 6.0 | 6.0 | 6.0 |
| | Ballast flow rate ($m^3$/Hr) | > 1,700 | Not determined | Not determined | Not determined |
| | Sample flow rate ($m^3$/hour) | 4 ± 10 % | 4.0 | 4.0 | 4.0 |
| | Pressure housing A (bar) | No requirement | 1.18 | 1.21 | 1.11 |
| | Pressure differential housing A (bar) | < 0.3 | - 0.02 | 0.05 | 0.08 |
| | Pressure housing B (bar) | No requirement | 1.33 | 1.35 | 1.26 |
| | Pressure differential housing B (bar) | < 0.3 | - 0.04 | 0.03 | 0.06 |
| | Drip sample volume (L) | No requirement | 11.5 | 12.0 | 12.0 |
| | Drip sample flow rate (L/Hr) | No requirement | 7.7 | 8.0 | 8.0 |
| **Test Cycle** | **Parameter** | **Valid Range** | **Tank 3P (Treatment)** | **Tank 4P (Treatment)** | **Tank 5P (Mock-Treatment)** |
| 2 | Sampling duration (min) | ≤ 90 | 78 | 81 | 90 |
| | Volume sampled ($m^3$)[10] | 5.4 -6.6 | 5.3* | 5.4 | 6.0 |
| | Ballast flow rate ($m^3$/Hr) | >1700 | 1,723 | 1,943 | 2,016 |
| | Sample flow rate ($m^3$/hour) | 3.6 – 4.4 | 4.0 | 4.0 | 4.0 |
| | Pressure housing A (bar) | No requirement | 0.96 | 0.96 | 1.05 |
| | Pressure differential housing A (bar) | < 0.30 | 0.06 | 0.07 | -0.04 |
| | Pressure housing B (bar) | No requirement | 1.19 | 1.21 | 1.30 |
| | Pressure differential housing B (bar) | < 0.30 | 0.14 | 0.17 | 0.07 |
| | Drip sample volume (L) | 40.5 – 49.5 | 42 | 43 | 45 |
| | Drip sample flow rate (L/Hr) | 27 - 33 | 32 | 32 | 30 |

[9] Based on assumption that p3SFS flow meter was accurately recording flow rates.
[10] As above.

Table 11. Summary of p3SFS operating conditions during Project 41012 discharge sampling events. Values marked with an asterisk (*) are outside the valid range for that parameter (Continued).

| Test Cycle | Parameter | Valid Range | Tank 3P (Treatment) | Tank 4P (Treatment) | Tank 5P (Mock-Treatment) |
|---|---|---|---|---|---|
| 3 | Sampling duration (min) | ≤ 90 | 89 | 68 | 90 |
| | Volume sampled ($m^3$)[11] | 1.6 – 10.4 (Target = 6) | 6.01 | 4.57 | 6.02 |
| | Ballast flow rate ($m^3$/Hr) | ≥ 1,700 | 1,520* | 1,792 | 1,820 |
| | Sample flow rate ($m^3$/hour) | 1 – 7 (Target = 4) | 4.01 | 4.03 | 4.01 |
| | Pressure differential housing A (psi) | ≤ 5 | - 0.21 | - 0.45 | - 0.31 |
| | Pressure differential housing B (psi) | ≤ 5 | 0.60 | 0.26 | 0.73 |
| | Drip sample volume (L) | 35 – 50 (Target = 45) | 49 | 50 | 48 |
| | Drip sample flow rate (L/Hr) | 23 – 33 (Target = 30) | 33 | 44* | 32 |
| **Test Cycle** | **Parameter** | **Valid Range** | **Tank Volume Equivalent #1** | **Tank Volume Equivalent #2** | **Tank Volume Equivalent #3** |
| 4 | Sampling duration (min) | 75 | 75 | 72 | Aborted due to unexpected change in ship ballast operations |
| | Volume sampled ($m^3$)[12] | 1.6 – 10.4 (Target = 6) | 5.1 | 4.7 | |
| | Ballast flow rate ($m^3$/Hr) | ≥ 1,700 | 6,284[13] | Could not determine; too few tank heights recorded. | |
| | Sample flow rate ($m^3$/hour) | 1 – 7 (Target = 4) | 4.1 | 3.9 | |
| | Average differential pressure (psi) | ≤ 5 | 1.2 | 1.5 | |
| | Drip sample volume (L) | 10 – 19 (Target = 15) | 4* | 3* | |
| | Drip sample flow rate (L/Hr) | 9 – 15 (Target = 12) | 3.2* | 2.5* | |

[11] As above.
[12] Based on assumption that p3SFS flow meter was accurately recording flow rates.
[13] On previous visits the IH ballasted a single tank at a time but in TC 4 they ballasted multiple tanks at once.

### 3.2.3 Proportionality of Sample Flow to Ballast Flow

The sample flow-ballast flow ratio could not be estimated as described in 2.4.1 because the p3SFS flow sensor was found to be inaccurate. The sensor problem was discovered at the end of the study when the sensor was compared to a reference flow sensor. The cause was traced to interference from the flow control valve located a short distance upstream of the flow sensor. Turbulence from the valve produced inaccurate sample flow data, which in turn affected flow control valve settings. The actual p3SFS system flow rates are unknown. (Appendix A). However, the test still provided useful results regarding the proportionality of a pre-programmed steady sample flow rate to a calculated actual ballast flow rate. GSI was able to assess how proportional a p3SFS preset target sample flow rate would have been to an actual ballast flow rate under real world conditions of uneven ballast flow rates.

Target volumes sampled and actual volume ballasted relative to TC2 and TC3[14] roughly corresponded only when ballast flow was not interrupted (Figures 12–15). For example, during TC2, recorded (i.e., target) sample flow was not proportional to calculated tank ballast flow when the IH ballasted ~500 m$^3$ of water in tank 5P before GSI sample collection started at 21:05 (Figure 12). The proportionality of the recorded sample flow to actual ballast flow was also affected during TC3 tank 4P discharge, where water from the tank was first used to flush the line (Figure 15).

[14] The proportionality of flow was determined after TC2 and TC3 by calculating the IH ballast flow using tank height data.

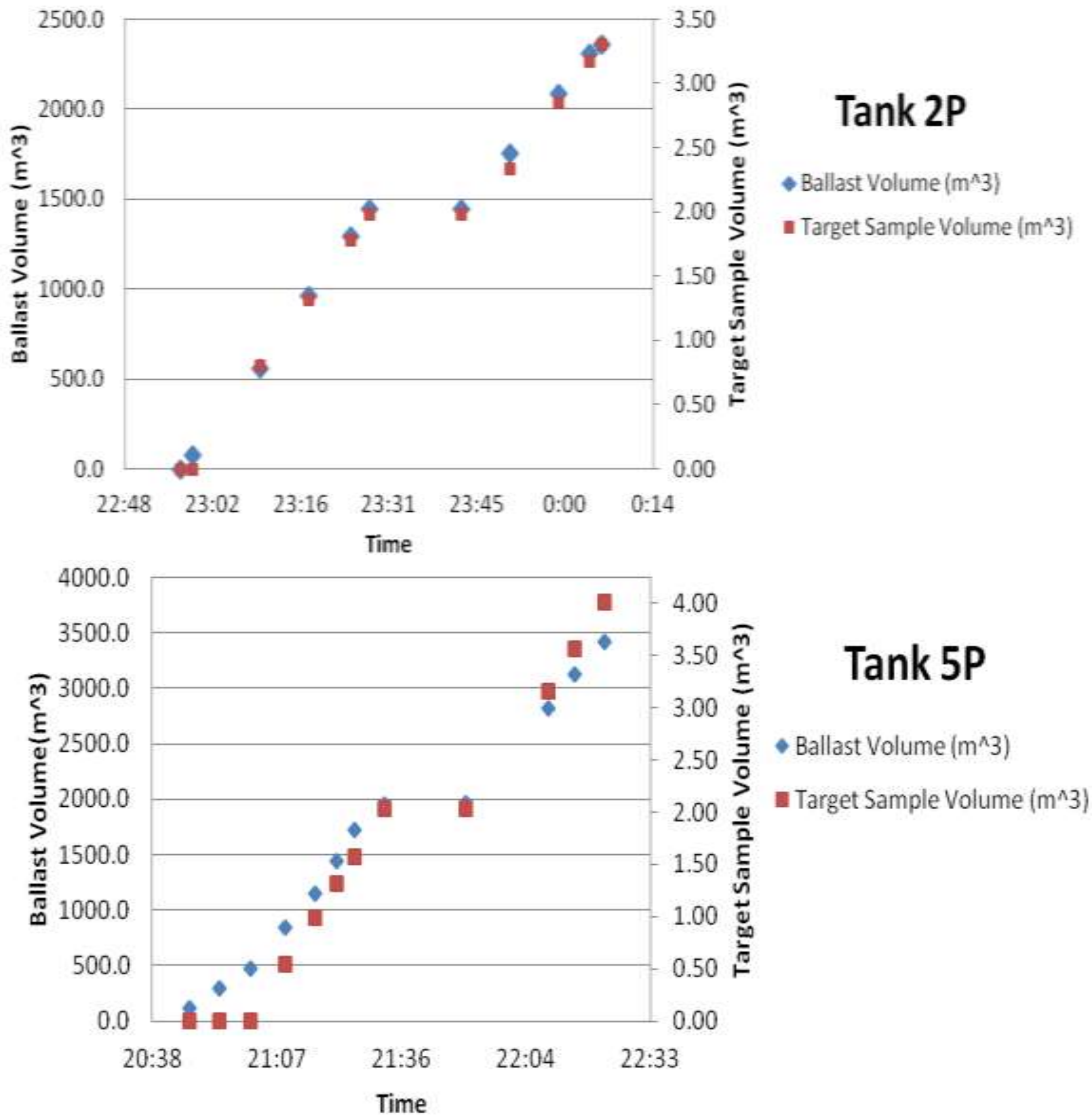
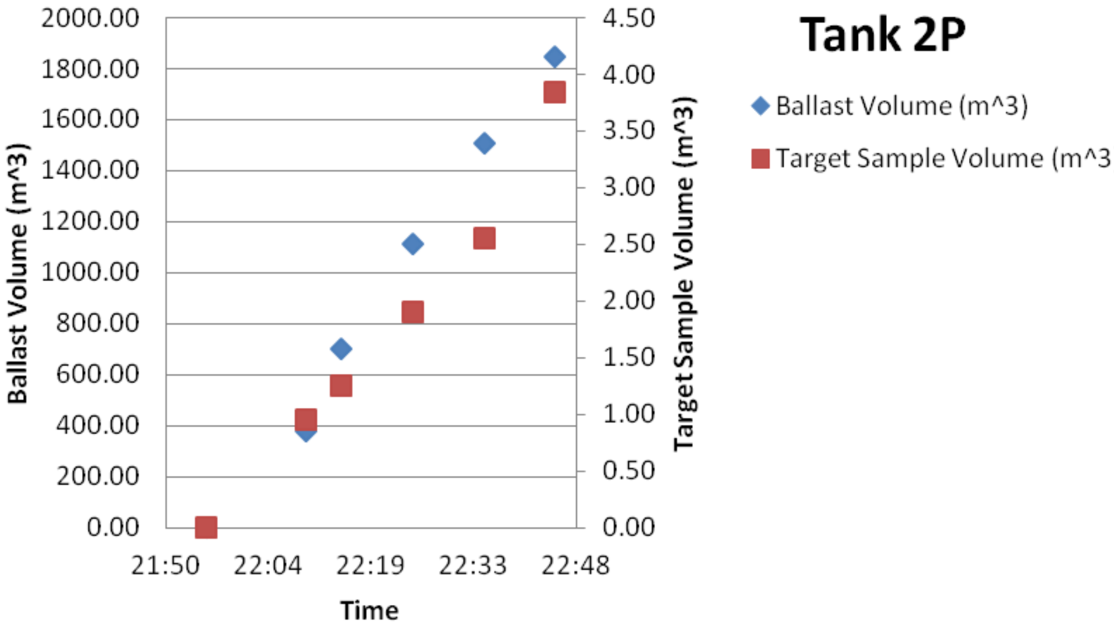



Figure 12. Calculated rate of ballast water loaded into tanks 2P and 5P during Test Cycle 2 intake operations compared to the target rate of sample water collected using the p3SFS[15].

[15] Based on assumption that p3SFS flow meter was accurately recording flow rates.



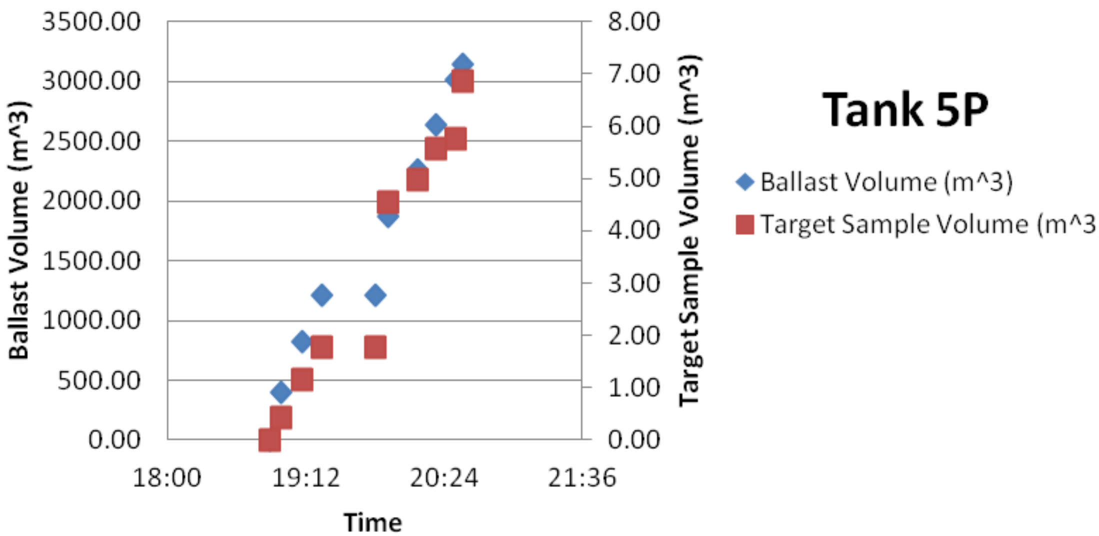




Figure 13. Calculated rate of ballast water loaded into tanks 2P and 5P during Test Cycle 3 intake operations compared to the target rate of sample water collected using the p3SFS16.

[16] Based on assumption that p3SFS flow meter was accurately recording flow rates.

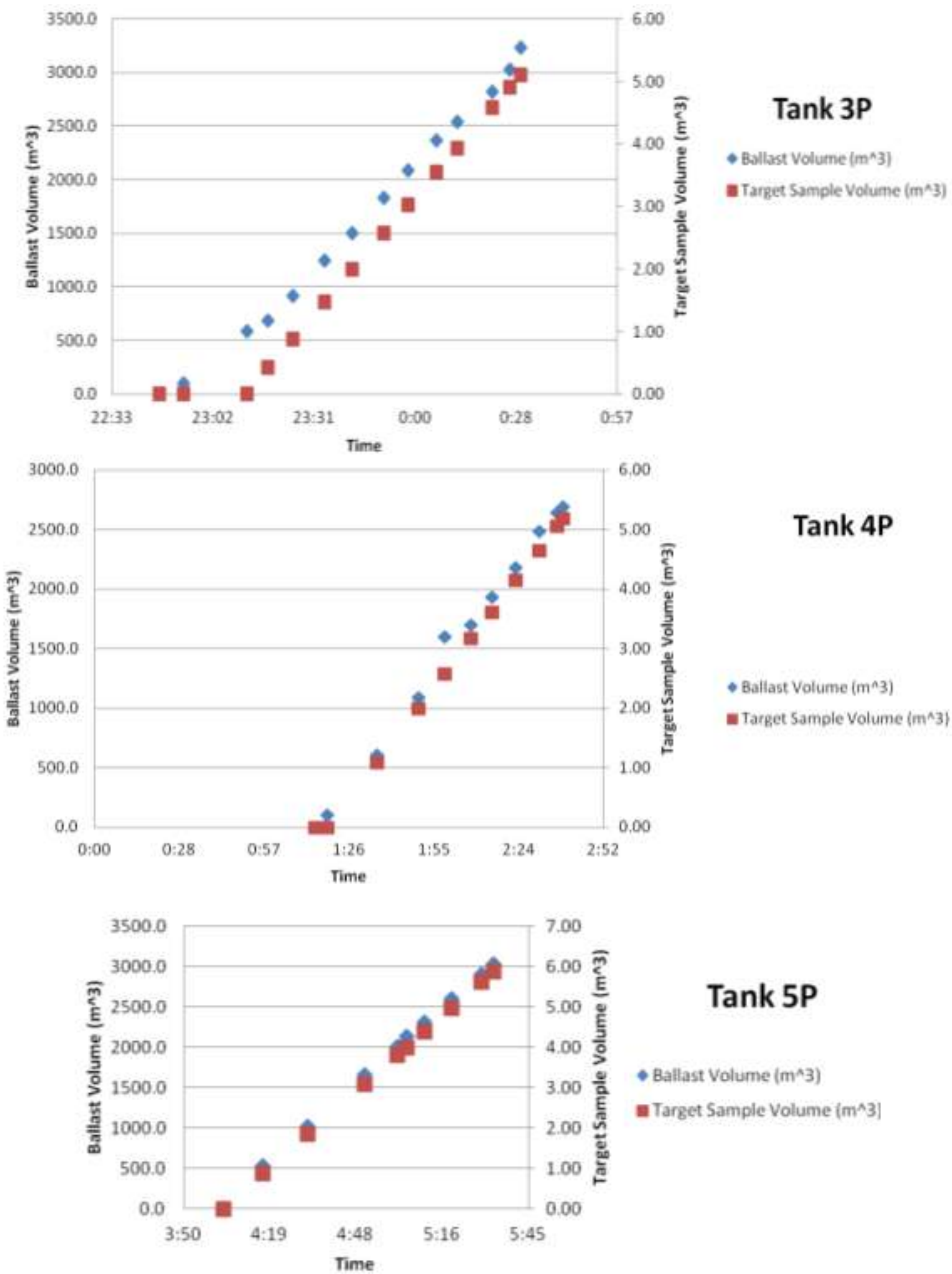



Figure 14. Estimated volume of ballast water discharged from tanks 3P, 4P and 5P during Test Cycle 2 discharge operations compared volume of sample water collected using the p3SFS17.

[17] Based on assumption that p3SFS flow meter was accurately recording flow rates.

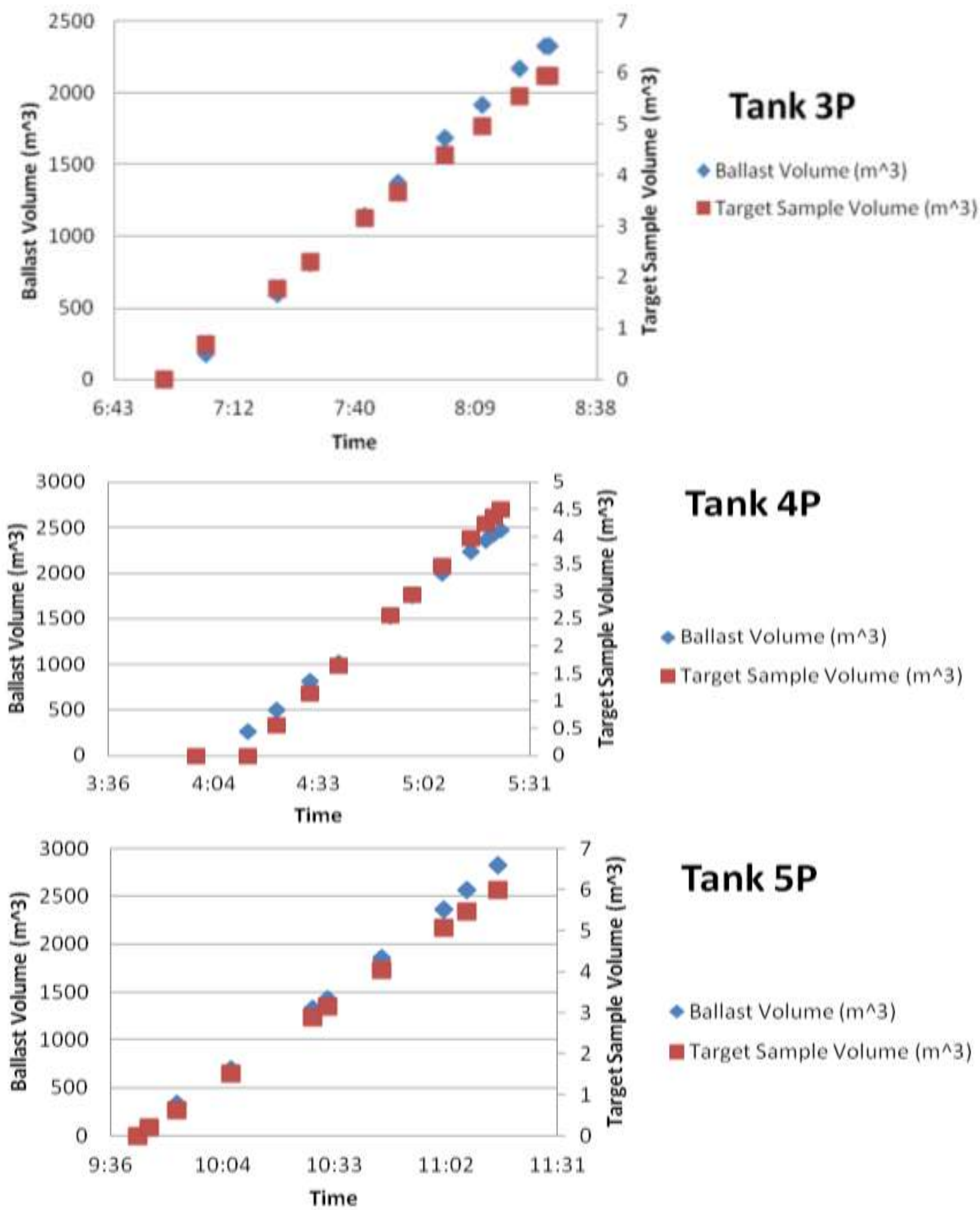



Figure 15. Estimated volume of water discharged from tanks 3P, 4P and 5P during Test Cycle 3 discharge operations compared volume of sample water collected using the p3SFS18.

[18] Based on assumption that p3SFS flow meter was accurately recording flow rates.

## 3.3 Characterization of Ballast Water Sampled in Test Cycles 1-4

### 3.3.1 Total Suspended Solids, Particulate Organic Matter, Percent Transmittance and Mineral Matter

#### *3.3.1.1 Intake*

Results from TC 1-4 analysis of intake samples for TSS, POM (TC 2-4 only), %T (filtered and unfiltered) and MM are presented in Table 12, and Figures 16-20. Across TCs, TSS was primarily inorganic MM (Table 12). For TC1, intake water chemistry challenge targets were met except for TSS (Table 12). For TC2, intake water chemistry challenge targets were met except for POM (Table 12). For TC3, intake water chemistry challenge targets were met except for TSS in tank 2P, measured from the only replicate available for analysis (Table 12). For TC4, where discrete grab samples were collected from the main ballast line instead of from the drip sampler, all intake water chemistry challenge targets were met (Table 12).

#### *3.3.1.2 Discharge*

Results from TC 1-3 analysis of discharge samples for TSS, POM (TC 2-4 only), %T (filtered and unfiltered) and MM are presented in Table 13 and Figures 16-20. No water chemistry analysis was conducted during TC4 because the sampling and analysis plan was reduced. Across TCs 1–3 however, concentrations of TSS and MM ranged from 4.4 mg/L to below the MDL (Table 13; Figures 16 and 20).

### 3.3.2 Non-Purgeable Organic Carbon, Dissolved Organic Carbon and Particulate Organic Carbon

#### *3.3.2.1 Intake*

Results from TC 1-4 analysis of intake samples for NPOC, DOC and POC are presented in Table 12, and Figures 21-23. Across TCs, TOC measured as NPOC was predominately in the form of DOC (Table 12).

#### *3.3.2.2 Discharge*

Results from TC 1-3 analysis of discharge samples for NPOC, DOC and POC are presented in Table 13 and Figures 21-23. No water chemistry analysis was conducted during TC4 owing to a truncated sampling and analysis plan. Across TCs 1–3, TOC concentrations (measured as NPOC) ranged from 2.7 mg/L to 6.7 mg/L (Table 13; Figure 21).

Table 12. Water chemistry parameters (Average ± Standard Deviation) measured from discrete grab samples collected during Test Cycles 1–4 Intake Operations. Values marked with an asterisk (*) are outside the valid range for that parameter.

| Test Cycle | Parameter | Challenge Target Valid Range | Tank 5P | Tank 2P | Tank 3P |
|---|---|---|---|---|---|
| 1 | Total Suspended Solids (mg/L) | ≥ 12 | 7.6 ± 0.2* | 5.3 ± 0.2* | 4.4 ± 0.1* |
| | Percent Transmittance - Filtered | No requirement | 90.7 ± 0.1 | 90.3 ± 0.9 | 90.4 ± 0.8 |
| | Percent Transmittance - Unfiltered | No requirement | 86.4 ± 0.2 | 86.1 ± 0.6 | 87.2 ± 0.8 |
| | Non-Purgeable Organic Carbon (mg/L) | No requirement | 3.2 ± 0.1 | 3.3 ± 0.1 | 3.1 ± 0.1 |
| | Dissolved Organic Matter – as Dissolved Organic Carbon (mg/L) | ≥ 2 | 2.9 ± 0.2 | 2.9 ± 0.1 | 2.8 ± 0.1 |
| | Particulate Organic Carbon (mg/L) | No requirement | 0.2 ± 0.1 | 0.4 ± 0.0 | 0.3 ± 0.0 |
| | Mineral Matter (mg/L) | No requirement | 7.3 ± 0.3 | 4.9 ± 0.2 | 4.1 ± 0.1 |
| **Test Cycle** | **Parameter** | **Challenge Target Valid Range** | **Tank 5P** | **Tank 2P** | |
| 2 | Total Suspended Solids (mg/L) | ≥ 12 | 20.3 ± 0.3 | 13.4 ± 0.6 | |
| | Percent Transmittance - Filtered | No requirement | 95.0 ± 0.6 | 94.9 ± 0.2 | |
| | Percent Transmittance - Unfiltered | No requirement | 84.1 ± 1.3 | 88.0 ± 0.3 | |
| | Non-Purgeable Organic Carbon (mg/L) | No requirement | 3.0 ± 0.3 | 2.7 ± 0.2 | |
| | Dissolved Organic Matter – as Dissolved Organic Carbon (mg/L) | ≥ 2 | 2.6 ± 0.2 | 2.6 ± 0.1 | |
| | Particulate Organic Carbon (mg/L) | No requirement | 0.5 ± 0.4 | 0.1 ± 0.2 | |
| | Particulate Organic Matter (mg/L) | ≥ 2 | 2.0 ± 0.1 | 1.4 ± 0.2* | |
| | Mineral Matter (mg/L) | No requirement | 19.9 ± 0.6 | 13.4 ± 0.8 | |
| **Test Cycle** | **Parameter** | **Challenge Target Valid Range** | **Tank 5P** | **Tank 2P** | |
| 3 | Total Suspended Solids (mg/L) | ≥ 12 | 16.9 ± 0.3 | 9.3* | |
| | Percent Transmittance - Filtered | No requirement | 61.9 ± 1.3 | 61.5 | |
| | Percent Transmittance - Unfiltered | No requirement | 53.2 ± 0.6 | 55.7 | |
| | Non-Purgeable Organic Carbon (mg/L) | No requirement | 7.4 ± 0.1 | 7.0 ± 0.1 | |
| | Dissolved Organic Matter – as Dissolved Organic Carbon (mg/L) | ≥ 2 | 6.5 ± 0.1 | 6.4 ± 0.2 | |
| | Particulate Organic Carbon (mg/L) | No requirement | 0.9 ± 0.1 | 0.6 ± 0.1 | |
| | Particulate Organic Matter (mg/L) | > 2 | 4.3 ± 0.1 | 3.3 | |
| | Mineral Matter (mg/L) | No requirement | 12.5 ± 0.2 | 6.0 | |
| **Test Cycle** | **Parameter** | **Challenge Target Valid Range** | **Tank Volume Equivalent #1** | **Tank Volume Equivalent #2** | **Tank Volume Equivalent #3** |
| 4 | Total Suspended Solids (mg/L) | ≥ 12 | 76.9 ± 4.3 | 70.0 ± 0.1 | 40.7 ± 8.5 |
| | Percent Transmittance - Filtered | No requirement | 58.0 ± 1.2 | 57.3 ± 0.1 | 55.6 ± 0.3 |
| | Percent Transmittance - Unfiltered | No requirement | 32.0 ± 1.7 | 33.2 ± 0.6 | 41.4 ± 1.5 |
| | Non-Purgeable Organic Carbon (mg/L) | No requirement | 6.2 ± 0.1 | 6.3 ± 0.1 | 6.2 ± 0.1 |
| | Dissolved Organic Matter – as Dissolved Organic Carbon (mg/L) | ≥ 2 | 6.4 ± 0.1 | 6.4 ± 0.1 | 6.6 ± 0.1 |
| | Particulate Organic Matter (mg/L) | ≥ 2 | 6.2 ± 0.5 | 5.7 ± 0.1 | 3.3 ± 0.8 |
| | Mineral Matter (mg/L) | No requirement | 70.6 ± 4.0 | 64.3 ± 0.0 | 37.4 ± 7.7 |

Table 13. Water chemistry parameters (Average ± Standard Deviation) measured from discrete grab samples collected during Test Cycles 1–4 discharge operations. MDL = Method Detection Limit.

| Test Cycle | Parameter | Tank 2P | Tank 3P | Tank 5P |
|---|---|---|---|---|
| 1 | Total Suspended Solids (mg/L) | 1.3 ± 0.2 | < MDL | < MDL |
| | Percent Transmittance - Filtered | 85.5 ± 0.2 | 87.5 ± 0.2 | 85.6 ± 0.3 |
| | Percent Transmittance - Unfiltered | 83.9 ± 0.3 | 86.0 ± 0.1 | 84.1 ± 0.1 |
| | Non-Purgeable Organic Carbon (mg/L) | 3.4 ± 0.1 | 3.2 ± 0.1 | 3.5 ± 0.3 |
| | Dissolved Organic Carbon (mg/L) | 3.2 ± 0.2 | 3.1 ± 0.1 | 3.3 ± 0.2 |
| | Particulate Organic Carbon (mg/L) | 0.2 ± 0.2 | 0.1 ± 0.1 | 0.2 ± 0.4 |
| | Mineral Matter (mg/L) | 1.1 ± 0.2 | 0.8 ± 0.1 | 0.8 ± 0.4 |
| Test Cycle | Parameter | Tank 3P (Treatment) | Tank 4P (Treatment) | Tank 5P |
| 2 | Total Suspended Solids (mg/L) | 2.3 ± 0.2 | 4.3 ± 0.2 | 3.7 ± 0.1 |
| | Percent Transmittance - Filtered | 90.3 ± 0.2 | 90.2 ± 0.5 | 90.8 ± 0.3 |
| | Percent Transmittance - Unfiltered | 89.3 ± 0.1 | 88.4 ± 0.3 | 85.5 ± 0.2 |
| | Non-Purgeable Organic Carbon (mg/L) | 2.8 ± 0.2 | 2.7 ± 0.1 | 2.9 ± 0.0 |
| | Dissolved Organic Carbon (mg/L) | 2.6 ± 0.0 | 2.8 ± 0.2 | 2.7 ± 0.1 |
| | Particulate Organic Carbon (mg/L) | 0.1 ± 0.2 | 0.0 ± 0.1 | 0.2 ± 0.1 |
| | Particulate Organic Matter (mg/L) | 0.5 ± 0.1 | 0.4 ± 0.2 | < MDL |
| | Mineral Matter (mg/L) | 2.2 ± 0.4 | 4.4 ± 0.1 | 3.5 ± 0.2 |
| Test Cycle | Parameter | Tank 3P (Treatment) | Tank 4P (Treatment) | Tank 5P |
| 3 | Total Suspended Solids (mg/L) | 1.2 ± 0.1 | 3.0 ± 0.0 | 3.3 ± 0.2 |
| | Percent Transmittance - Filtered | 68.3 ± 0.3 | 68.1 ± 0.2 | 61.2 ± 0.2 |
| | Percent Transmittance - Unfiltered | 67.0 ± 0.2 | 67.0 ± 0.1 | 58.2 ± 0.1 |
| | Non-Purgeable Organic Carbon (mg/L) | 5.8 ± 0.1 | 6.0 ± 0.0 | 6.7 ± 0.1 |
| | Dissolved Organic Carbon (mg/L) | 5.8 ± 0.1 | 5.8 ± 0.2 | 6.2 ± 0.0 |
| | Particulate Organic Carbon (mg/L) | < MDL | < MDL | 0.5 ± 0.1 |
| | Particulate Organic Matter (mg/L) | < MDL | < MDL | 1.0 ± 0.3 |
| | Mineral Matter (mg/L) | 1.2 ± 0.1 | 3.0 ± 0.0 | 2.3 ± 0.4 |
| Test Cycle | Parameter | Tank Volume Equivalent #1 | Tank Volume Equivalent #2 | Tank Volume Equivalent #3 |
| 4 | Total Suspended Solids (mg/L) | No water chemistry samples collected due to truncated sampling plan. | | |
| | Percent Transmittance - Filtered | | | |
| | Percent Transmittance - Unfiltered | | | |
| | Non-Purgeable Organic Carbon (mg/L) | | | |
| | Dissolved Organic Carbon (mg/L) | | | |
| | Particulate Organic Matter (mg/L) | | | |
| | Mineral Matter (mg/L) | | | |

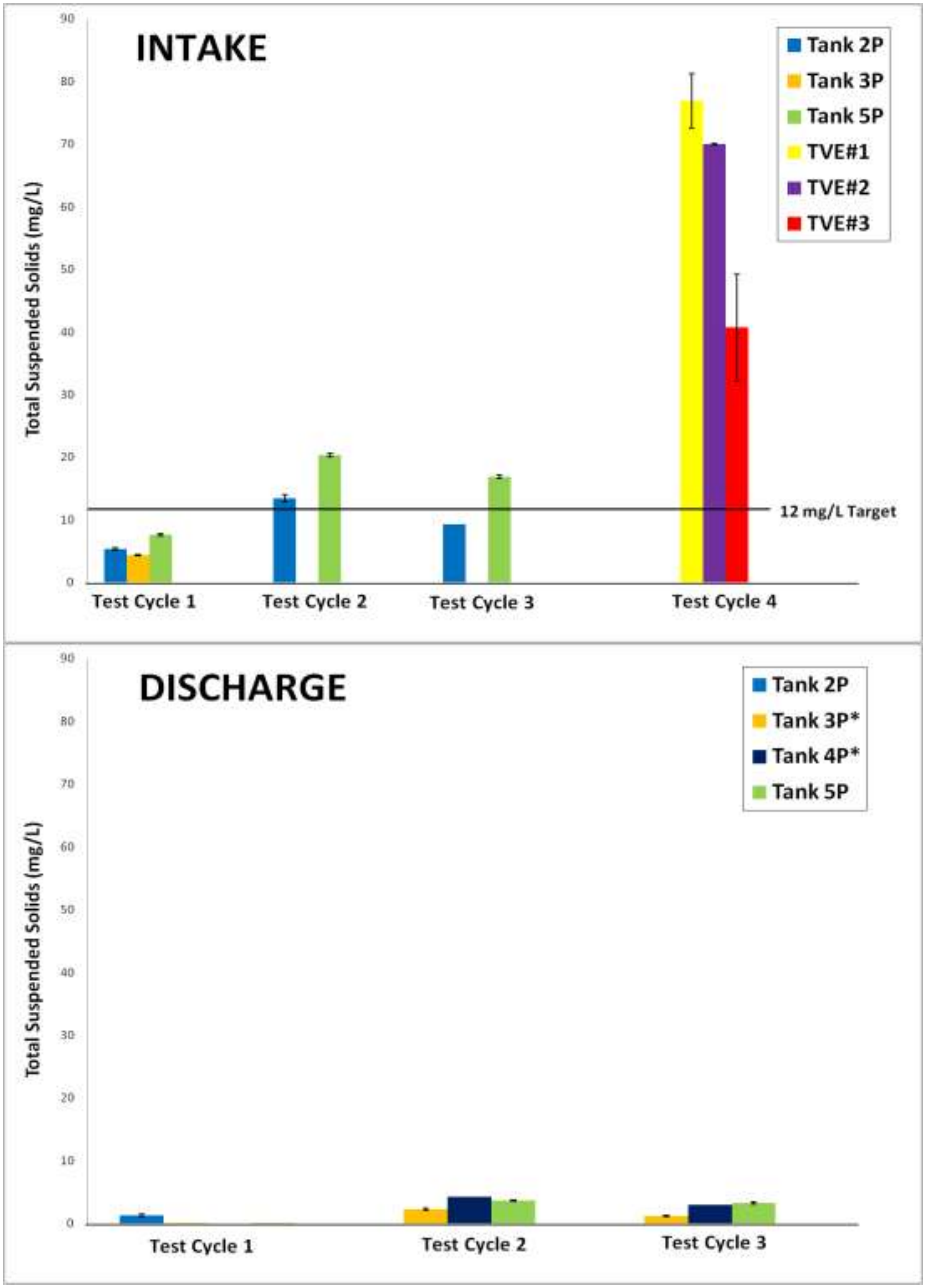



Figure 16. Test Cycle 1-4 intake and discharge concentrations of total suspended solids. *Tank 3P and 4P were treated during Test Cycles 2 and 3.

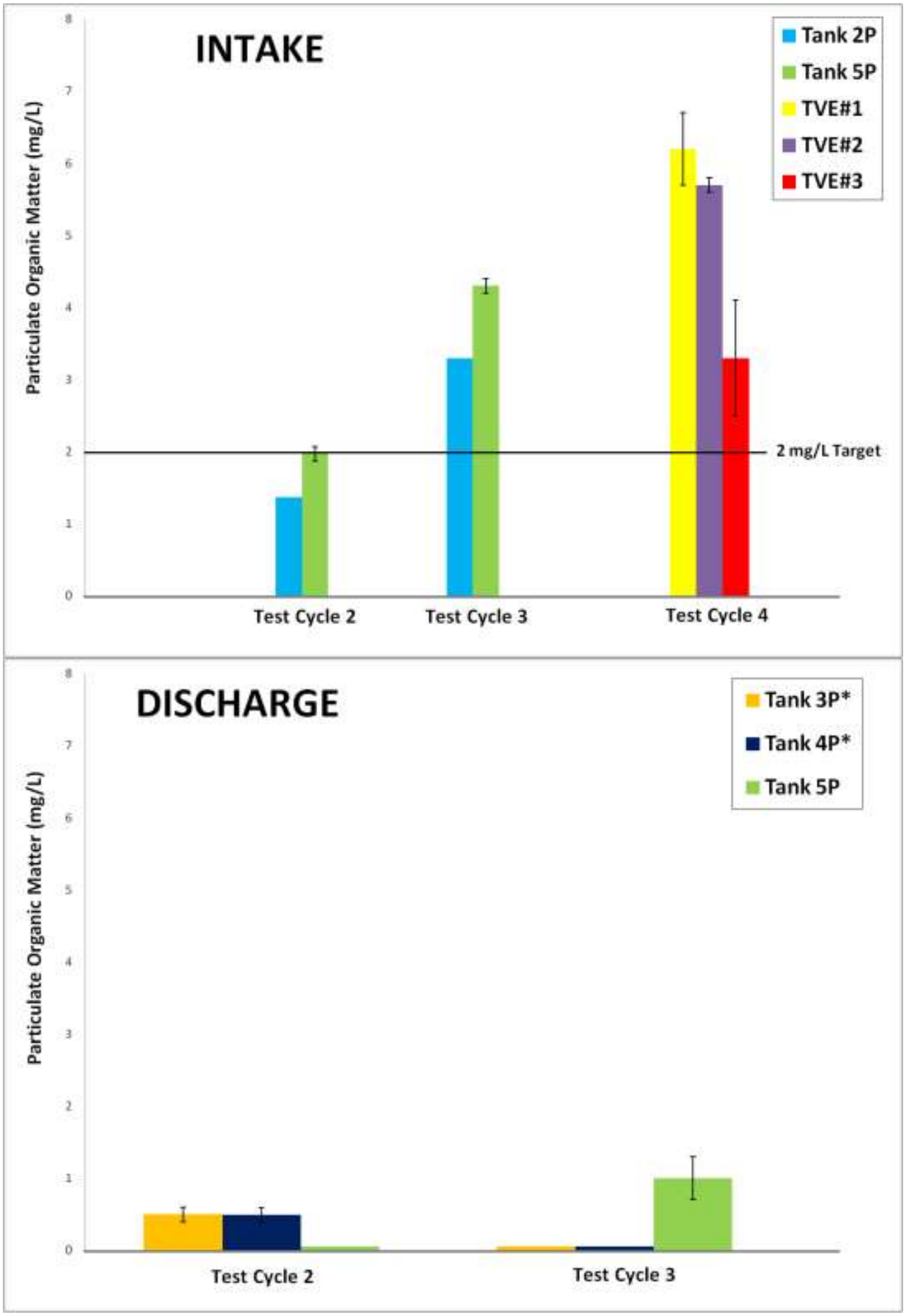
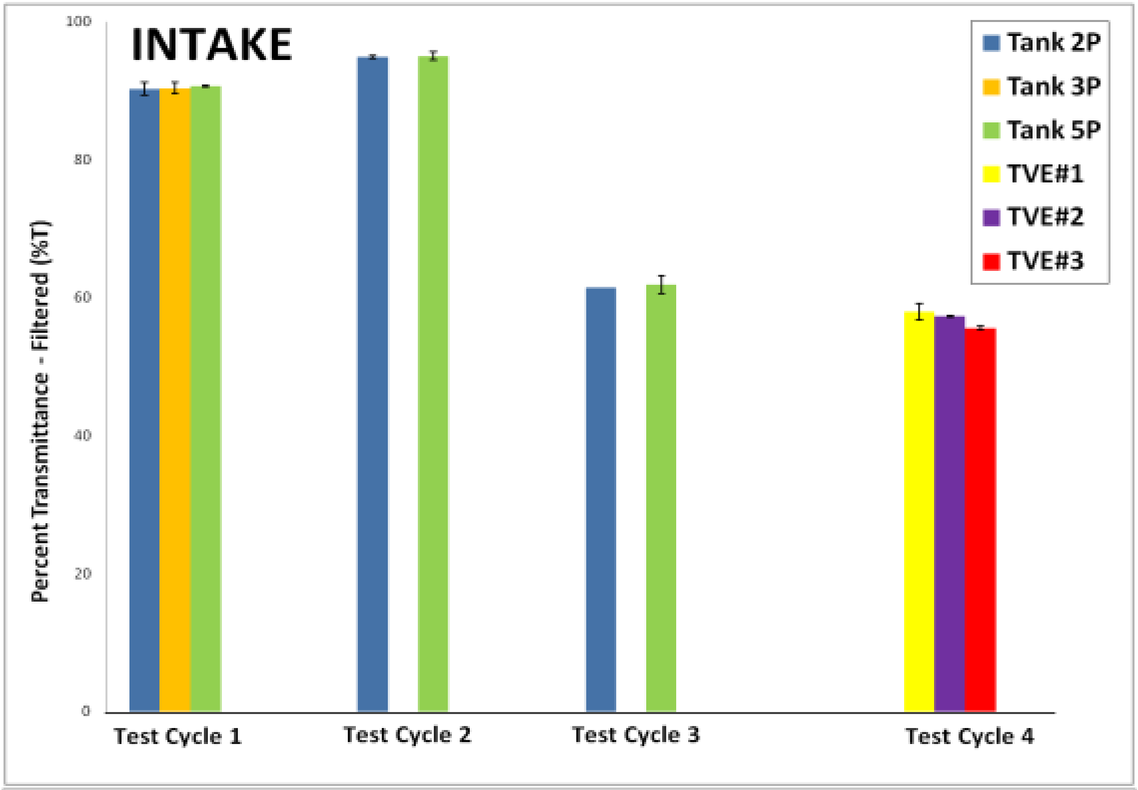



Figure 17. Test Cycle 1-4 intake and discharge concentrations of particulate organic matter. *Tank 3P and 4P were treated during Test Cycles 2 and 3.



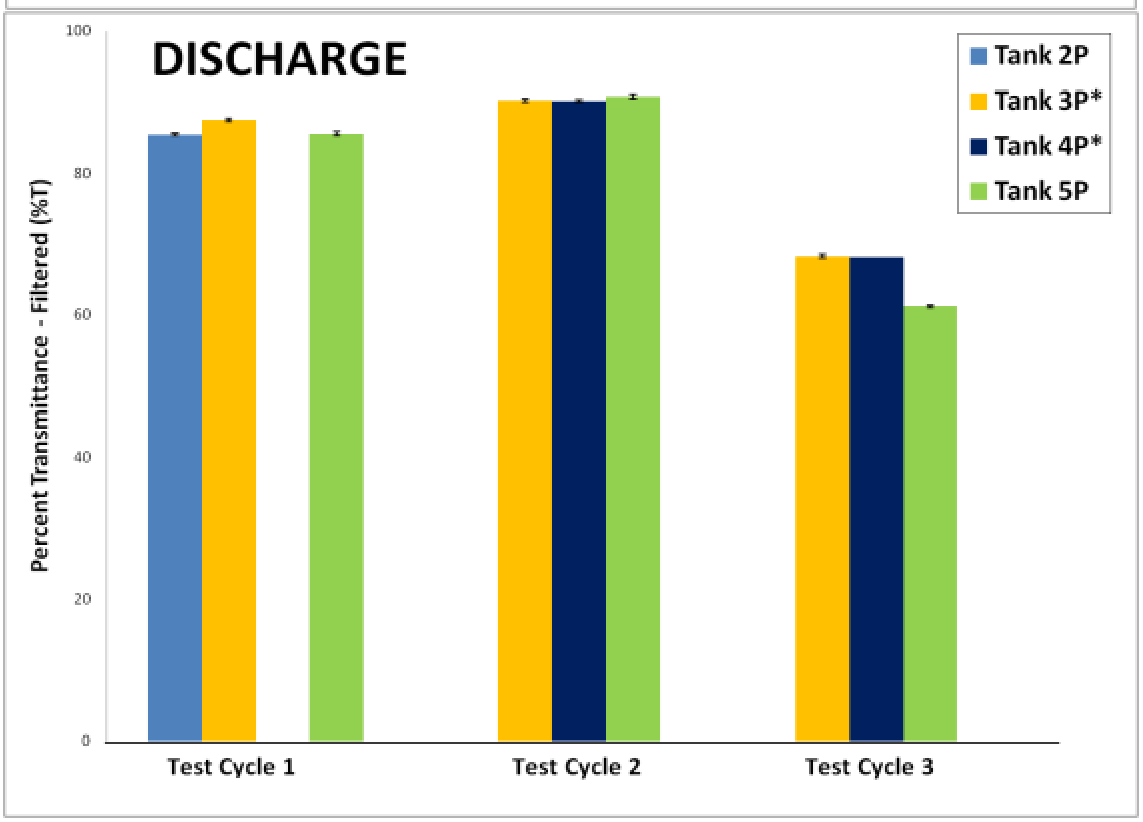




Figure 18. Test Cycle 1-4 intake and discharge concentrations of percent transmittance - filtered. *Tank 3P and 4P were treated during Test Cycles 2 and 3.

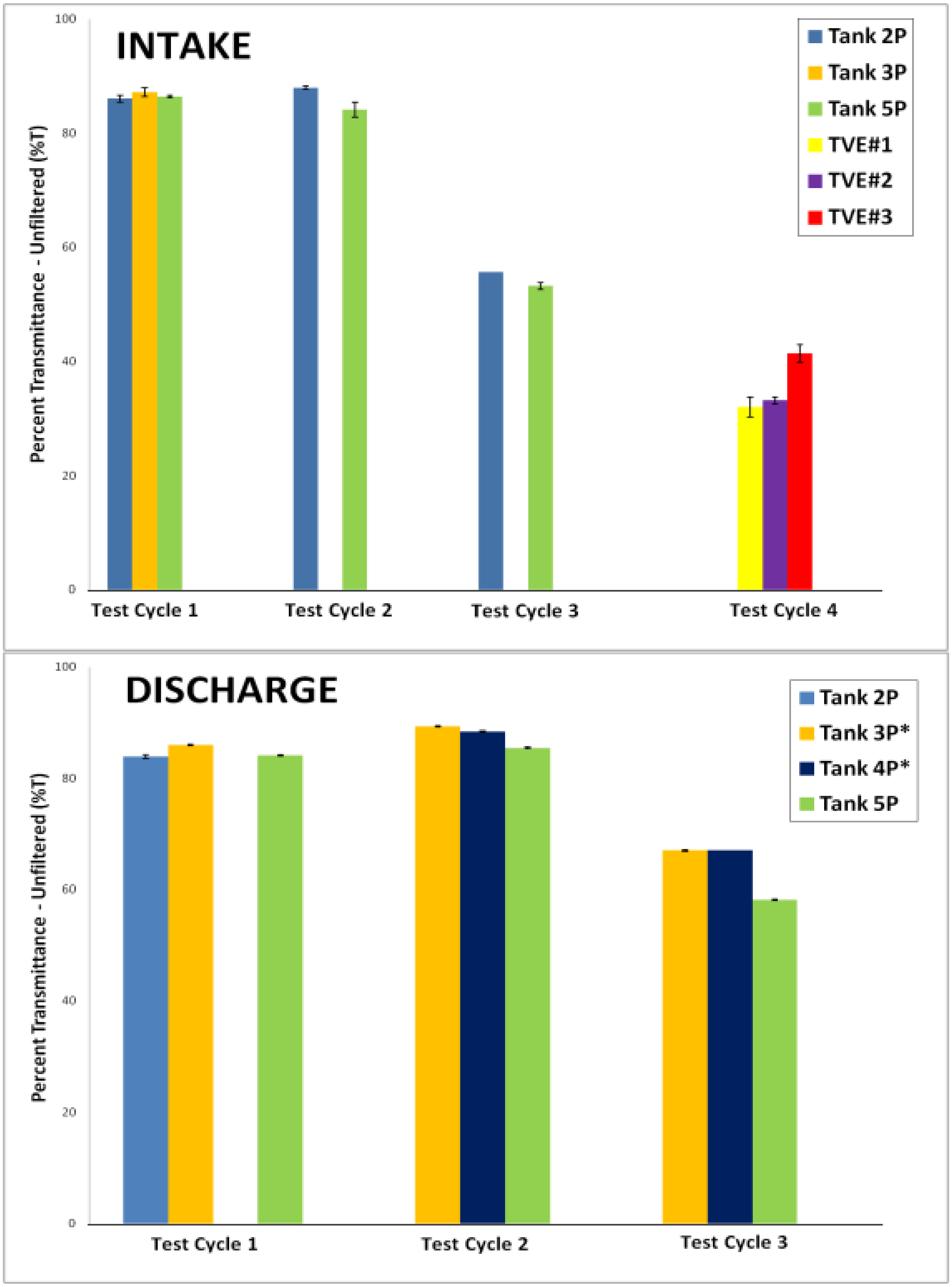



Figure 19. Test Cycle 1-4 intake and discharge concentrations of percent transmittance - unfiltered. *Tank 3P and 4P were treated during Test Cycles 2 and 3.

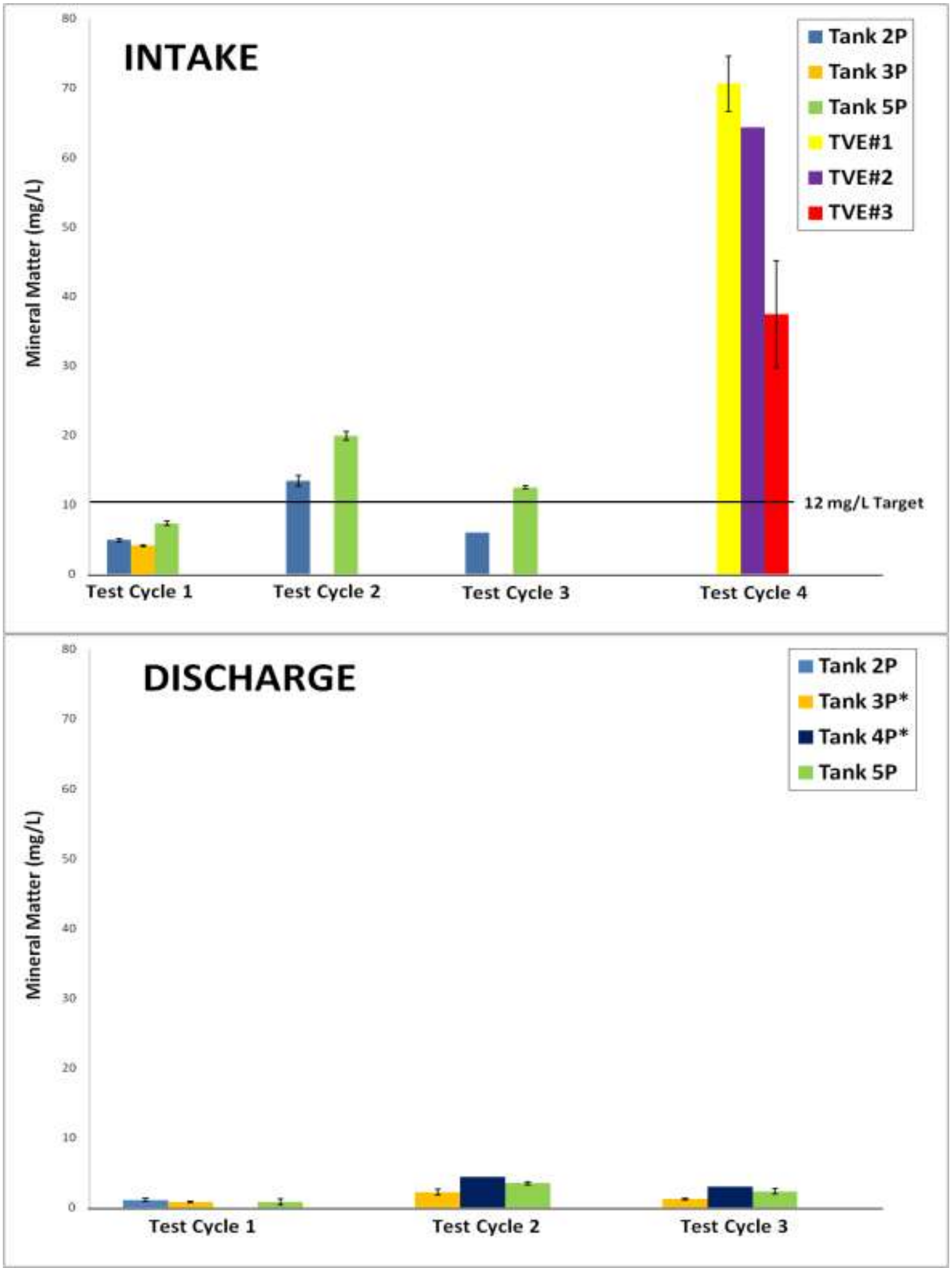



Figure 20. Test Cycle 1-4 intake and discharge concentrations of mineral matter. *Tank 3P and 4P were treated during Test Cycles 2 and 3.

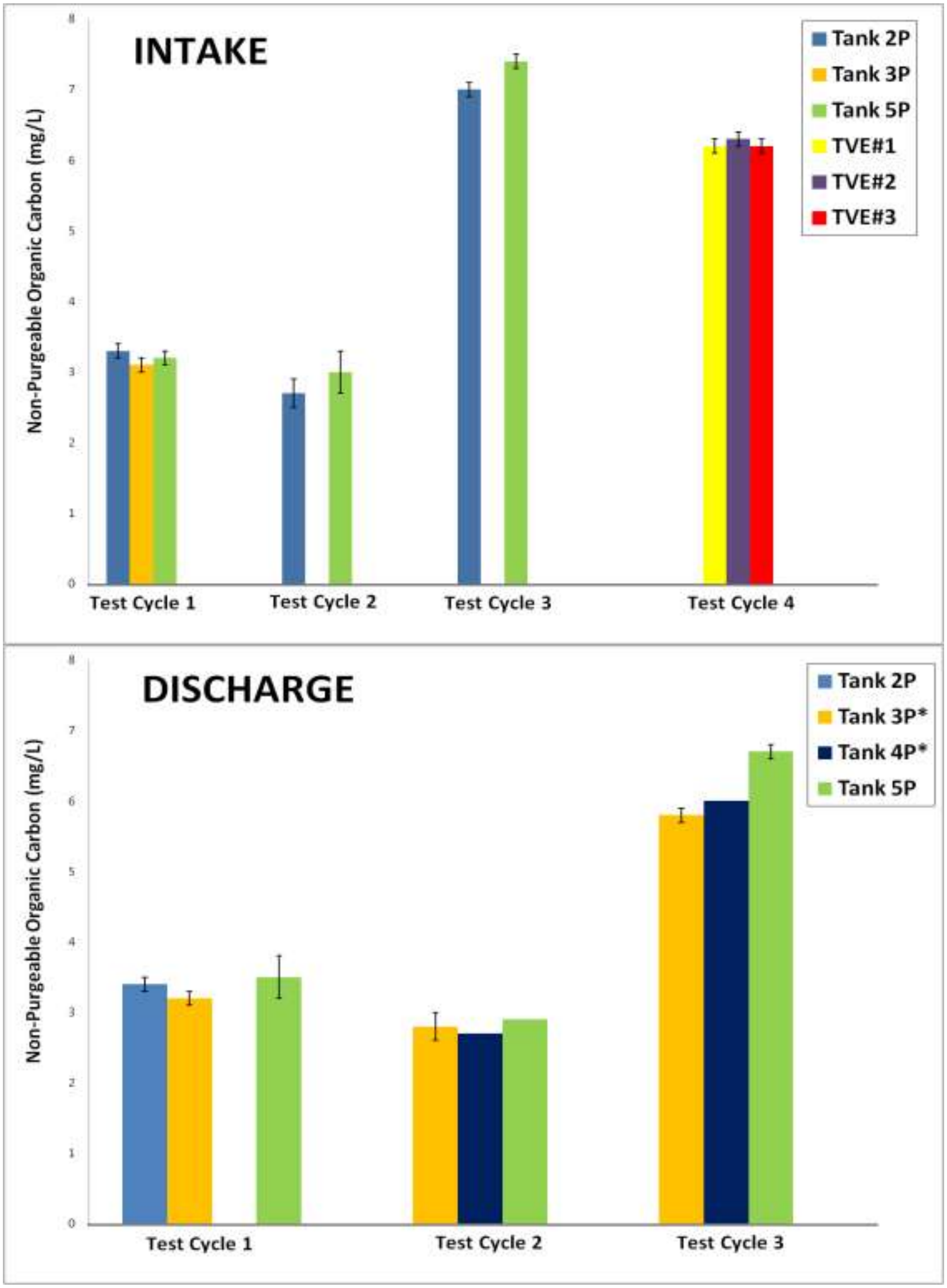



Figure 21. Test Cycle 1-4 intake and discharge concentrations of non-purgeable organic carbon. *Tank 3P and 4P were treated during Test Cycles 2 and 3.

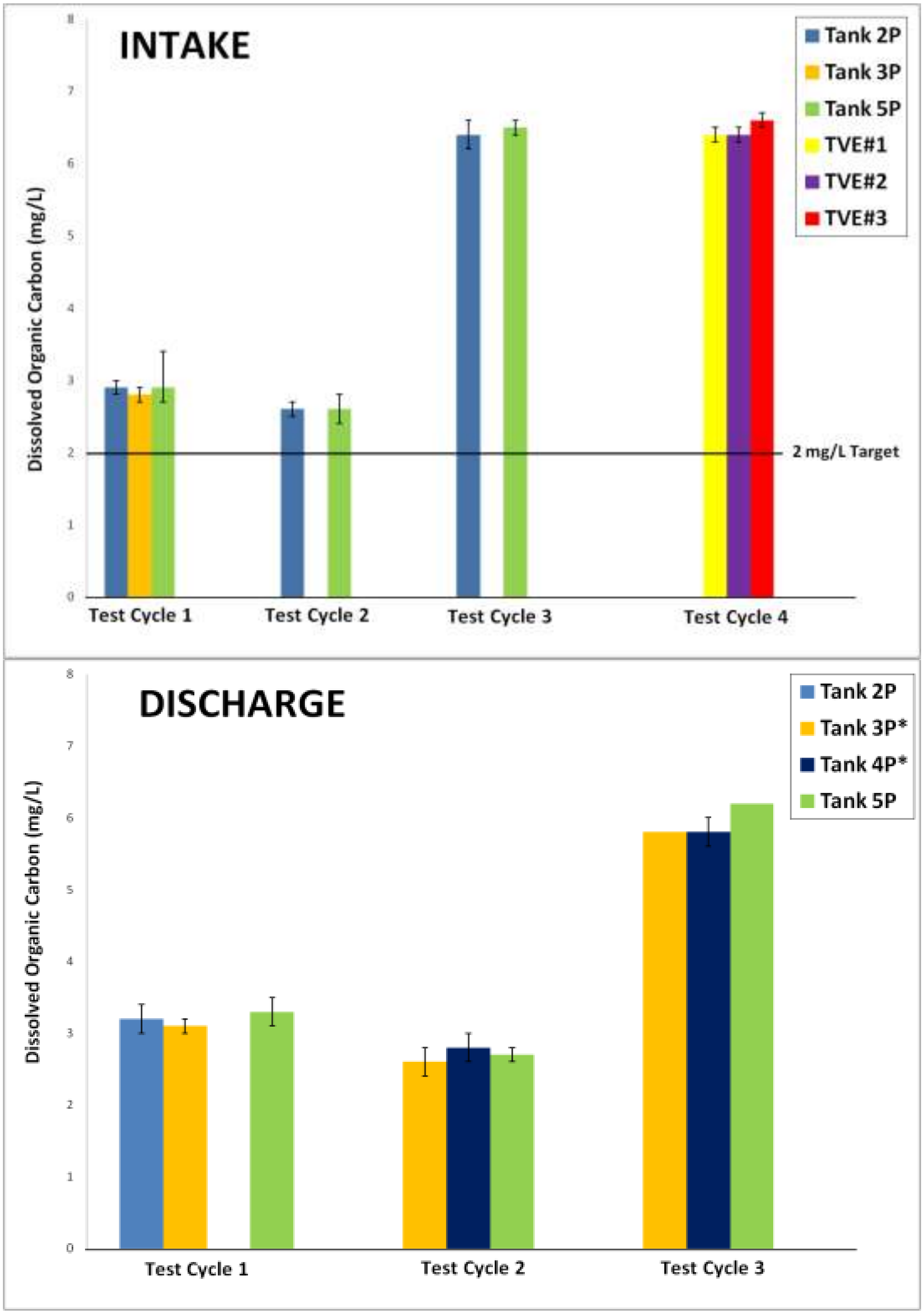



Figure 22. Test Cycle 1-4 intake and discharge concentrations of dissolved organic carbon. *Tank 3P and 4P were treated during Test Cycles 2 and 3.

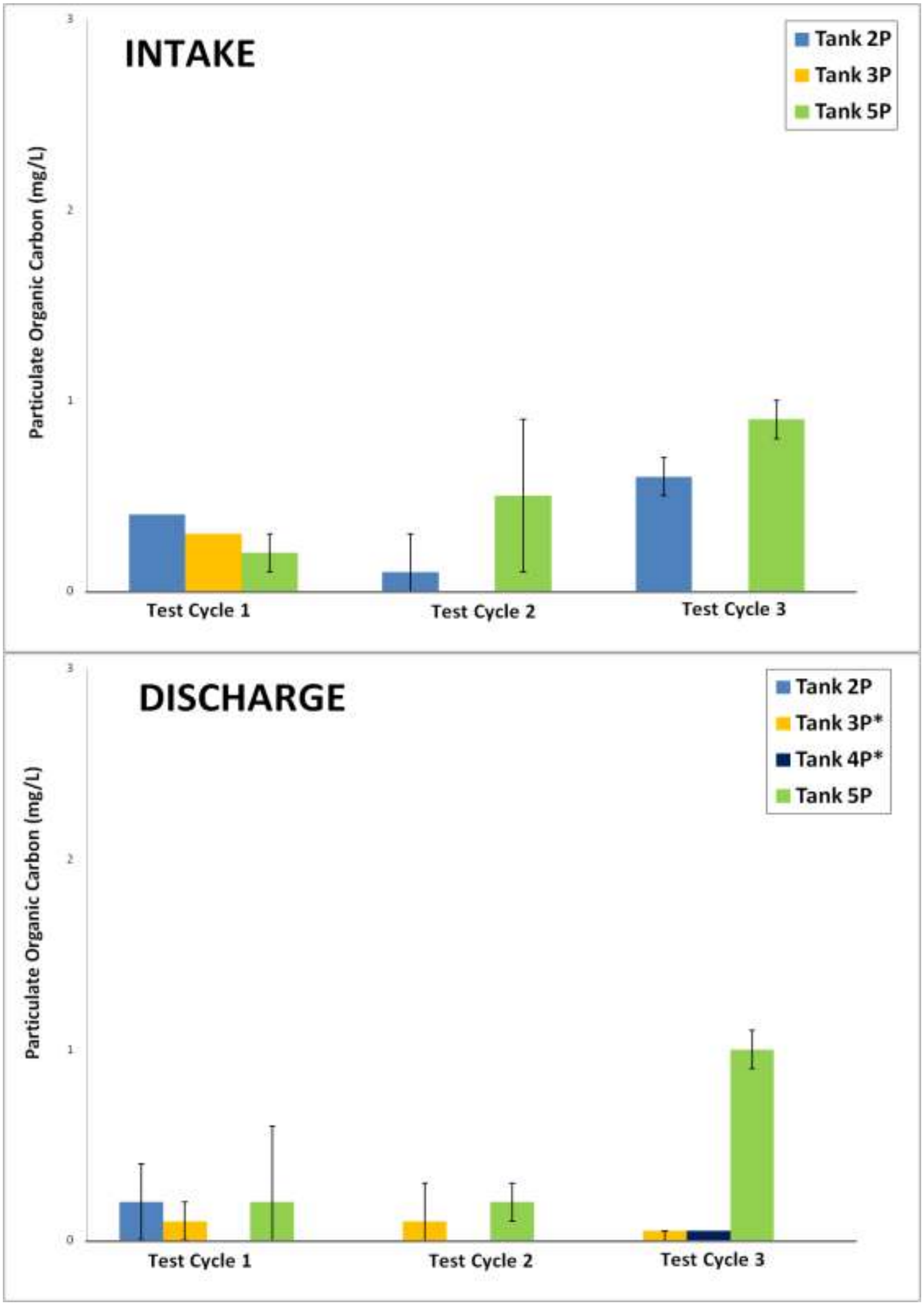



Figure 23. Test Cycle 1-4 intake and discharge concentrations of particulate organic carbon. *Tank 3P and 4P were treated during Test Cycles 2 and 3.

### 3.3.3 Other Water Quality Parameters

#### *3.3.3.1 Intake*

Table 14 and Figures 24–31 summarize TC 1-4 intake water quality parameters measured using calibrated YSI Multiparameter Water Quality Sondes (including temperature, specific conductivity, salinity, pH, turbidity, total chlorophyll and dissolved oxygen) and the p3SFS's in-line temperature and turbidity sensors (Figure 32). Across all TCs, where data were available, intake targets were met for temperature and salinity (Table 14). However, the temperature and turbidity data reported by the YSI Sondes and p3SFS in-line sensors are not comparable (Table 14; Figures 24 and 28). The p3SFS temperature sensor was either not functioning or was out of calibration. For TC2 tank 2P, Table 14 and Figure 24 only report p3SFS in-line sensor data for temperature after the ballasting pause because data measured prior to the pause were erased. For TC3, no readings were obtained from the p3SFS in-line turbidity sensor because the turbidity probe malfunctioned (Table 14; Figure 28). In addition, the specific conductivity and salinity data measured using YSI Sondes for the two tanks sampled during TC3 intake are erroneous and therefore not reported (Table 14; Figures 25 and 26). The root cause of this error is unknown, but may be due to a recording error, malfunction of the Sonde and/or a calibration issue.

#### *3.3.3.2 Discharge*

Table 15 and Figures 24–31 summarize TC 1-4 discharge water quality parameters measured using calibrated YSI Multiparameter Water Quality Sondes (including temperature, specific conductivity, salinity, pH, turbidity, total chlorophyll and dissolved oxygen) and the p3SFS's in-line sensors (temperature and turbidity only). There was no continuous, in-line electronic data available from the p3SFS' sensors for temperature or turbidity for TC1 discharge due to an error in the formatting of the SD card to which the electronic data were transferred. Values from the p3SFS control screen were recorded by hand and are provided in Table 15 and Figures 24 and 28. As with TC3 intake measurements, the specific conductivity and salinity data from the YSI Sondes for all three tanks sampled on discharge are erroneous and are not reported (Table 15; Figures 25 and 26). Similarly, for this TC, no p3SFS readings for turbidity were available because the turbidity probe had failed (Table 15; Figure 28).

Overall, temperature data measured by the p3SFS's in-line sensors and the YSI Sondes were somewhat consistent with each other, however the turbidity data were not (Table 15; Figures 24 and 28). For TCs 2 and 3, there were several differences between treated and untreated discharge water quality parameters, including salinity, turbidity and dissolved oxygen/percent saturation (Table 15; Figures 24, 25, 30 and 31).

Table 14. Water quality parameters (Average ± Standard Deviation) measured by YSI Multiparameter Sondes and the p3SFS in-line sensors during Test Cycles 1–4 intake operations.

| Parameter | Measurement Device | Challenge Target Valid Range | Test Cycle 1 | Test Cycle 2 | Test Cycle 3 | Test Cycle 4 |
|---|---|---|---|---|---|---|
| Temperature (˚C) | YSI Multiparameter Sonde | 2-35 | 31.36 ± 0.72 | 17.38 ± 0.08 | 25.95 ± 0.78 | 12.03 ± 1.06 |
| | p3SFS In-Line Sensor | | 24.0 ± 0.0 | 15.0 ± 0.2 | 20.6 ± 0.0 | 13.4 ± 0.5 |
| Specific Conductivity (mS/cm) | YSI Multiparameter Sonde | No requirement | 0.416 ± 0.003 | 0.325 ± 0.001 | Not reported | 0.129 ± 0.123 |
| Salinity (ppt) | YSI Multiparameter Sonde | < 1 (for freshwater) | 0.20 ± 0.01 | 0.16 ± 0.00 | Not reported | 0.06 ± 0.06 |
| pH | YSI Multiparameter Sonde | No requirement | 8.06 | 8.74 | 8.07 | 7.57 |
| Turbidity (NTU) | YSI Multiparameter Sonde | No requirement | 6.5 ± 1.8 | 12.6 ± 1.6 | 8.1 ± 2.1 | 45.5 ± 12.2 |
| | p3SFS In-Line Sensor | | 14.7 ± 9.3 | 90.5 ± 12.0 | No logged data | 205.3 ± 59.2 |
| Total Chlorophyll (μg/L) | YSI Multiparameter Sonde | No requirement | 10.0 ± 1.0 | 4.0 ± 0.2 | 3.8 ± 0.4 | 2.1 ± 0.3 |
| Dissolved Oxygen (% Saturation) | YSI Multiparameter Sonde | No requirement | 94.3 ± 0.2 | 76.2 ± 0.3 | 93.4 ± 1.3 | 85.3 ± 1.6 |
| Dissolved Oxygen (mg/L) | YSI Multiparameter Sonde | No requirement | 6.95 ± 0.07 | 7.27 ± 0.05 | 7.58 ± 0.21 | 9.14 ± 0.35 |

Table 15. Water quality parameters (Average ± Standard Deviation) measured by YSI Multiparameter Sondes and the p3SFS in-line sensors during Test Cycles 1–4 discharge operations.

| Parameter | Measurement Device | Test Cycle 1 | Test Cycle 2 | | Test Cycle 3 | | Test Cycle 4 |
|---|---|---|---|---|---|---|---|
| | | | Mock-Treatment (5P) | Treatment (3P and 4P) | Mock-Treatment (5P) | Treatment (3P and 4P) | |
| Temperature (˚C) | YSI Multiparameter Sonde | 25.21 ± 0.02 | 12.77 | 12.62 ± 0.29 | 19.91 | 21.01 ± 0.25 | 9.15 ± 0.47 |
| | p3SFS In-Line Sensor | 21.42 ± 0.2 | 12.8 ± 0.2 | 12.8 ± 0.1 | 18.2 ± 0.6 | 17.8 ± 0.0 | 11.8 ± 1.5 |
| Specific Conductivity (mS/cm) | YSI Multiparameter Sonde | 0.390 ± 0.003 | 0.327 | 0.787 ± 0.026 | Not reported | Not reported | 0.157 ± 0.064 |
| Salinity (ppt) | YSI Multiparameter Sonde | 0.19 ± 0.01 | 0.16 | 0.39 ± 0.01 | Not reported | Not reported | 0.08 ± 0.04 |
| pH | YSI Multiparameter Sonde | 8.05 | 8.01 | 8.03 | 7.75 | 7.78 | 7.86 |
| Turbidity (NTU) | YSI Multiparameter Sonde | 2.5 ± 0.3 | 4.3 | 3.7 ± 0.8 | 3.0 | 3.0 ± 0.6 | 14.1 ± 0.4 |
| | p3SFS In-Line Sensor | 9.7 ± 5.5 | 28 ± 14 | 38.5 ± 9.2 | No logged data | No logged data | 57.6 ± 2.1 |
| Total Chlorophyll (μg/L) | YSI Multiparameter Sonde | 4.3 ± 0.3 | 2.0 | 1.7 ± 0.1 | 1.8 | 1.3 ± 0.3 | 1.3 ± 0.1 |
| Dissolved Oxygen (% Saturation) | YSI Multiparameter Sonde | 94.8 ± 0.9 | 66.5 | 72.6 ± 1.8 | 70.1 | 77.9 ± 4.8 | 87.0 ± 4.1 |
| Dissolved Oxygen (mg/L) | YSI Multiparameter Sonde | 7.79 ± 0.07 | 7.01 | 7.69 ± 0.24 | 6.36 | 6.91 ± 0.40 | 10.00 ± 0.37 |

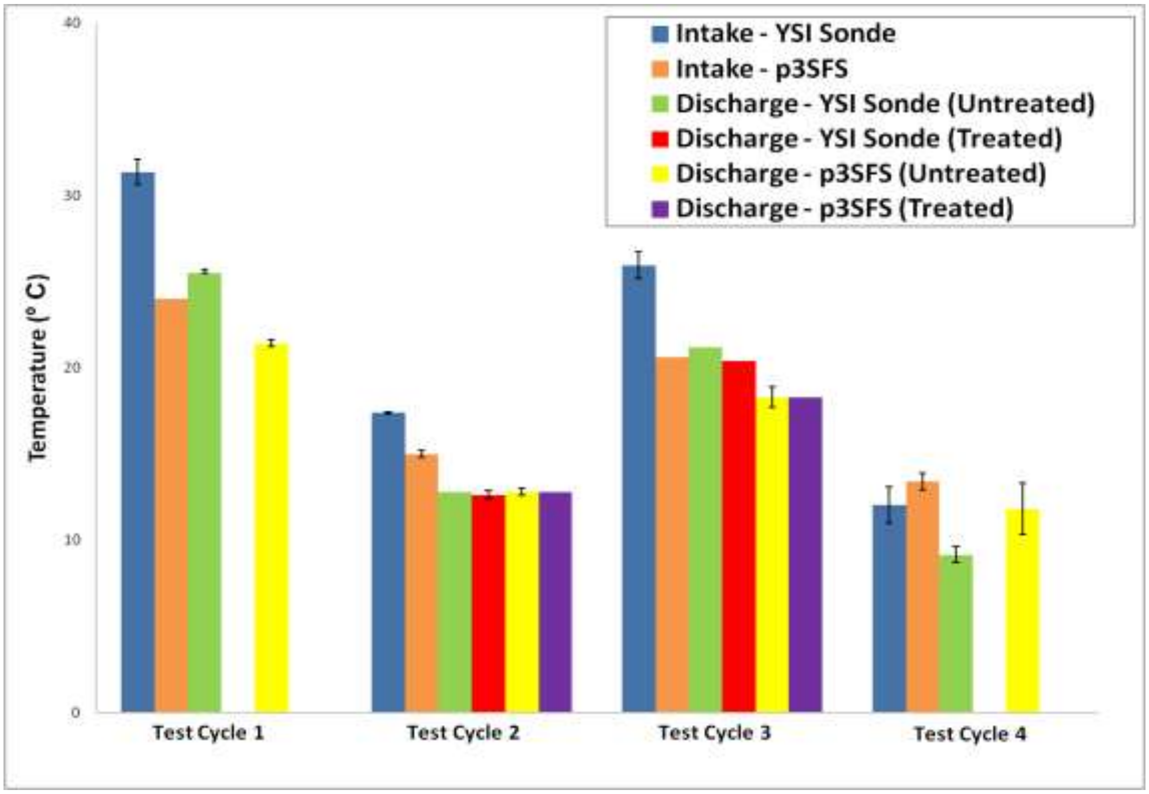



Figure 24. Test Cycle 1-4 intake and discharge temperature measurements (measured using a Multiparameter Sonde and the p3SFS in-line sensor).

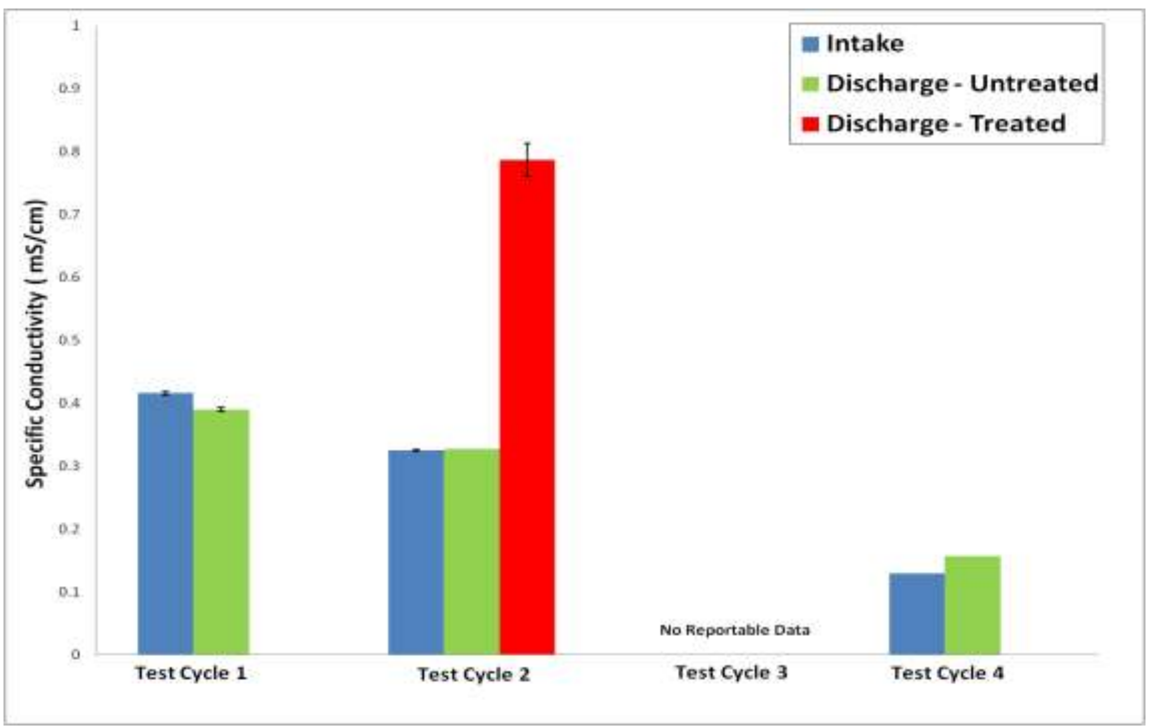



Figure 25. Test Cycle 1-4 intake and discharge specific conductivity measurements (measured using a YSI Multiparameter Sonde).

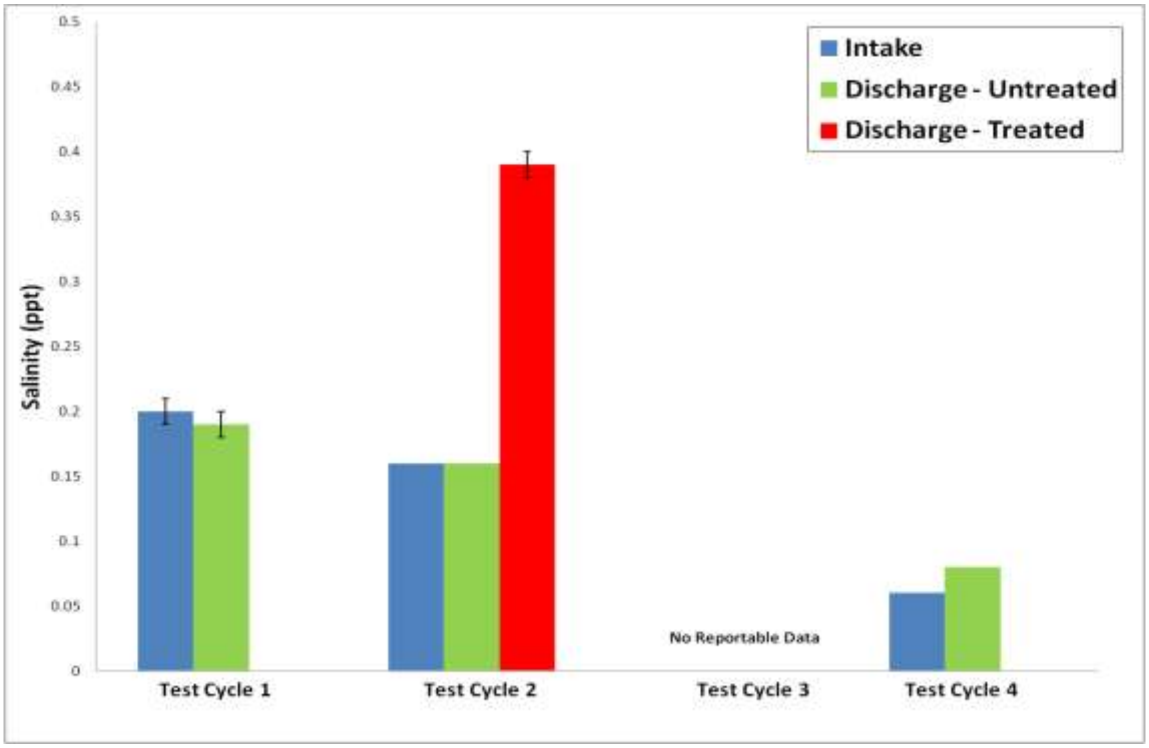



Figure 26. Test Cycle 1-4 intake and discharge salinity measurement (measured using a YSI Multiparameter Sonde).

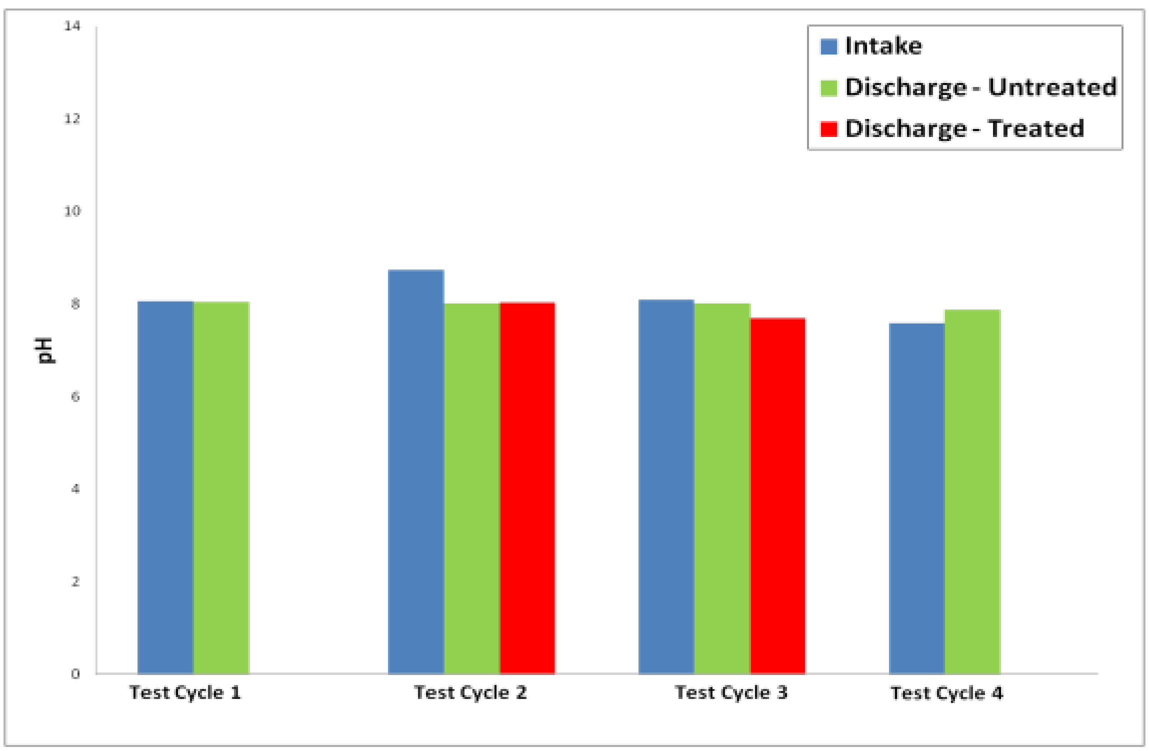



Figure 27. Test Cycle 1-4 intake and discharge pH measurements (measured using a YSI Multiparameter Sonde).

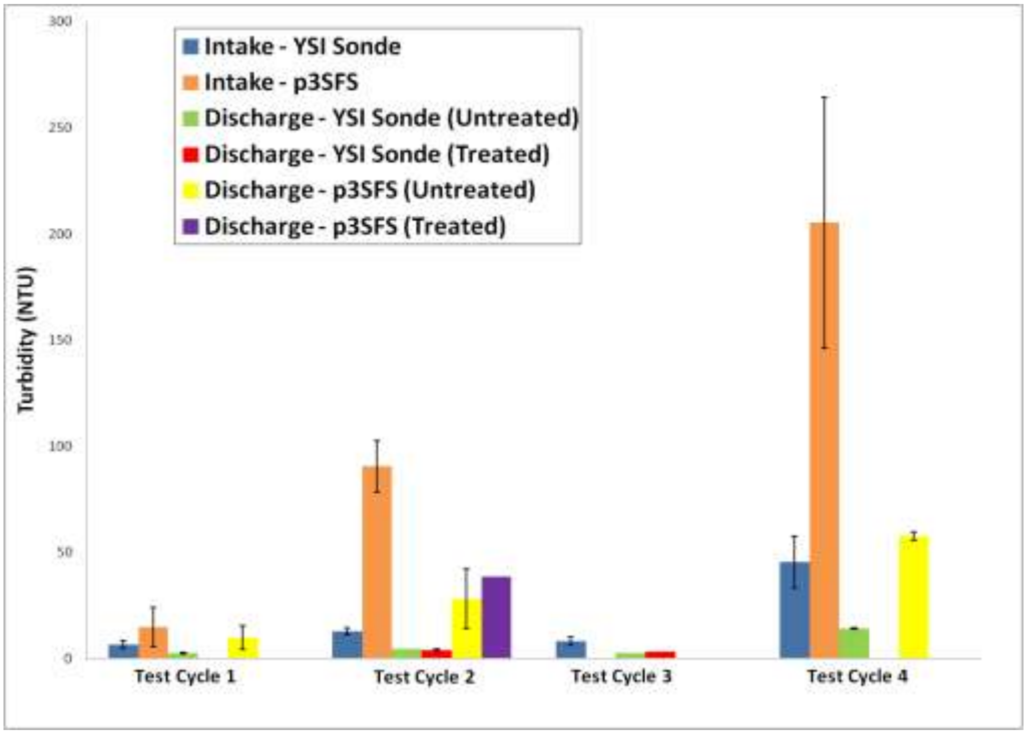



Figure 28. Test Cycle 1-4 intake and discharge turbidity measurements (measured using a Multiparameter Sonde and the p3SFS in-line sensor).

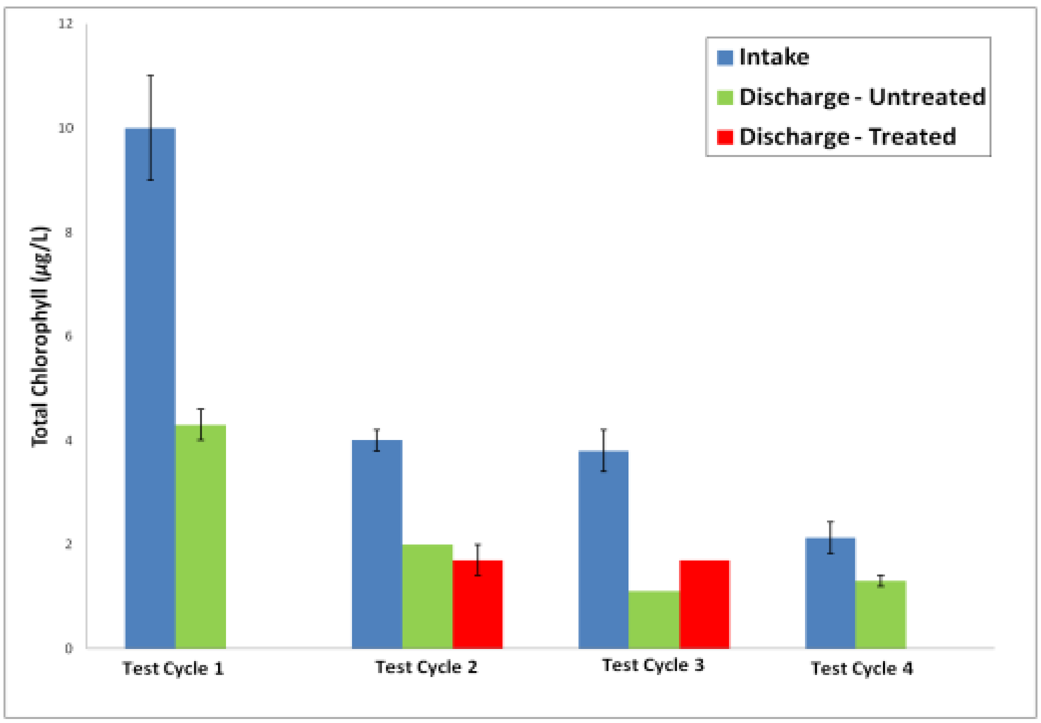



Figure 29. Test Cycle 1-4 intake and discharge total chlorophyll measurements (measured using a YSI Multiparameter Sonde).

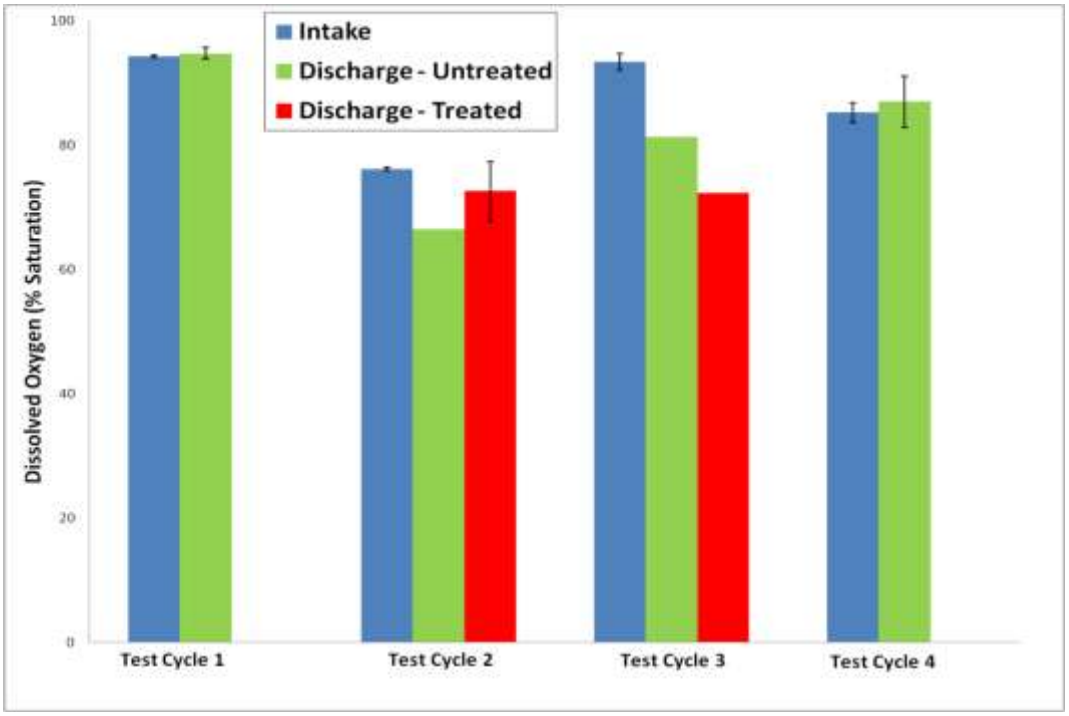



Figure 30. Test Cycle 1-4 intake and discharge dissolved oxygen (percent saturation) measurements (measured using a YSI Multiparameter Sonde).

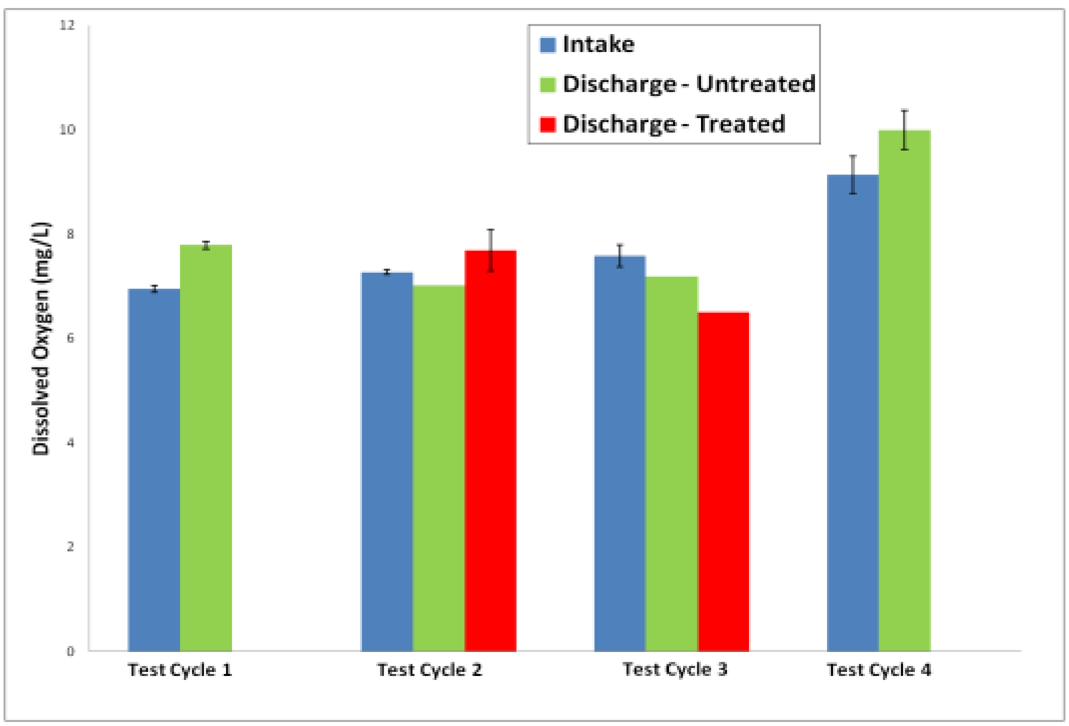



Figure 31. Test Cycle 1-4 intake and discharge dissolved oxygen measurements (measured using a YSI Multiparameter Sonde).


Figure 32. AquaSensors display on the p3SFS showing the in-line temperature and turbidity data in real time.

### 3.3.4 Biology

The densities of live zooplankton reported in this section are subject to error due to a malfunction in the p3SFS flow meter detected after the tests reported here were completed. All other values are based on whole water samples, and are therefore considered accurate.

#### ***3.3.4.1*** *Organisms ≥ 50 µm19*

The density of live organisms ≥ 50 $\mu$m in intake samples was above the challenge target of 10,000/m$^3$ for TCs 1–3 (Table 16; Figure 33). For TC4, the density of organisms in this size class exceeded the winter challenge target of 1,000/m$^3$ (Table 16; Figure 33). In all TCs the organisms ≥ 50 $\mu$m also met the requirements for challenge water diversity (Figure 34). Dominant taxa within the zooplankton community varied across TCs (Figure 35).

On discharge, live densities of organisms ≥ 50 $\mu$m in untreated discharge ranged from > 400,000/m$^3$ in TC3 to < 12,000 /m$^3$ in TC4 (Table 17 and Figure 33). In some cases overall densities, driven by rotifer reproduction, were higher in discharge than intake (Figure 35). In the treated tanks (TC2 and TC3), live organism densities declined by 87 % and 93 % compared to the intake densities, primarily due to the loss of rotifers (Figure 35).

[19] All densities based on assumption that p3SFS flow meter was accurately recording flow rates.

#### *3.3.4.2 Organisms ≥ 10 and < 50 µm*

The density of live organisms ≥ 10 $\mu$m and < 50 $\mu$m in intake samples was above the challenge target of 500 cells/mL for TCs 1 and 3 and below the challenge target for TCs 2 and 4 (Table 16; Figure 36). Although the community composition of organisms ≥ 10 $\mu$m and < 50 $\mu$m in intake samples varied across TCs, at least five species from at least three different phyla occurred in all intake samples, meeting target diversity challenge conditions.

On discharge, live organism densities in the ≥ 10 and < 50 $\mu$m size class are reported in Table 17 and Figure 36. A treatment effect was not detected in TC2, but in TC3 organism densities were one order of magnitude lower in treated versus untreated samples. Still, treated samples live organism densities exceeded the discharge standard by one order of magnitude (Table 17; Figure 36). Time-integrated discharge samples were collected during TC4 for analysis of organisms in the ≥ 10 and < 50 $\mu$m size class, but they were not analyzed because of the drip sampler malfunction.

#### *3.3.4.3 Organisms < 10 µm*

TCs 1-3 intake samples contained concentrations of culturable, aerobic heterotrophic bacteria which exceeded the minimum challenge condition of 500 per mL using the SimPlate® and spread plate analysis methods (Table 16; Figures 37 and 38). TC4 was truncated and no time-integrated samples were collected for analysis of organisms in the < 10 $\mu$m size class (Table 16; Figure 38). Densities of *E. coli*, total coliform bacteria and *Enterococcus* spp., i.e., fecal contamination indicator organisms, were generally moderate to low (Table 16; Figure 39-41).

Discharge densities of organisms < 10 $\mu$m are reported in Table 17 and Figures 37 to 41. Heterotrophic bacteria, as measured by the SimPlate® method, ranged from 490 MPN/mL to 153,000 MPN/mL during TCs 1–3, with treated and untreated samples during TC2 and TC3 not substantially different from one another (Table 17; Figure 37). In comparison, discharge heterotrophic bacteria densities measured by the spread plate method ranged from 935 CFU/mL to 31,900 CFU/mL; densities were slightly higher in untreated samples than treated samples during TC2 and TC3 (Table 17; Figure 38). Densities of *E. coli*, total coliform bacteria and *Enterococcus* spp., were extremely low on intake and (Table 17 and Figures 39 – 41).

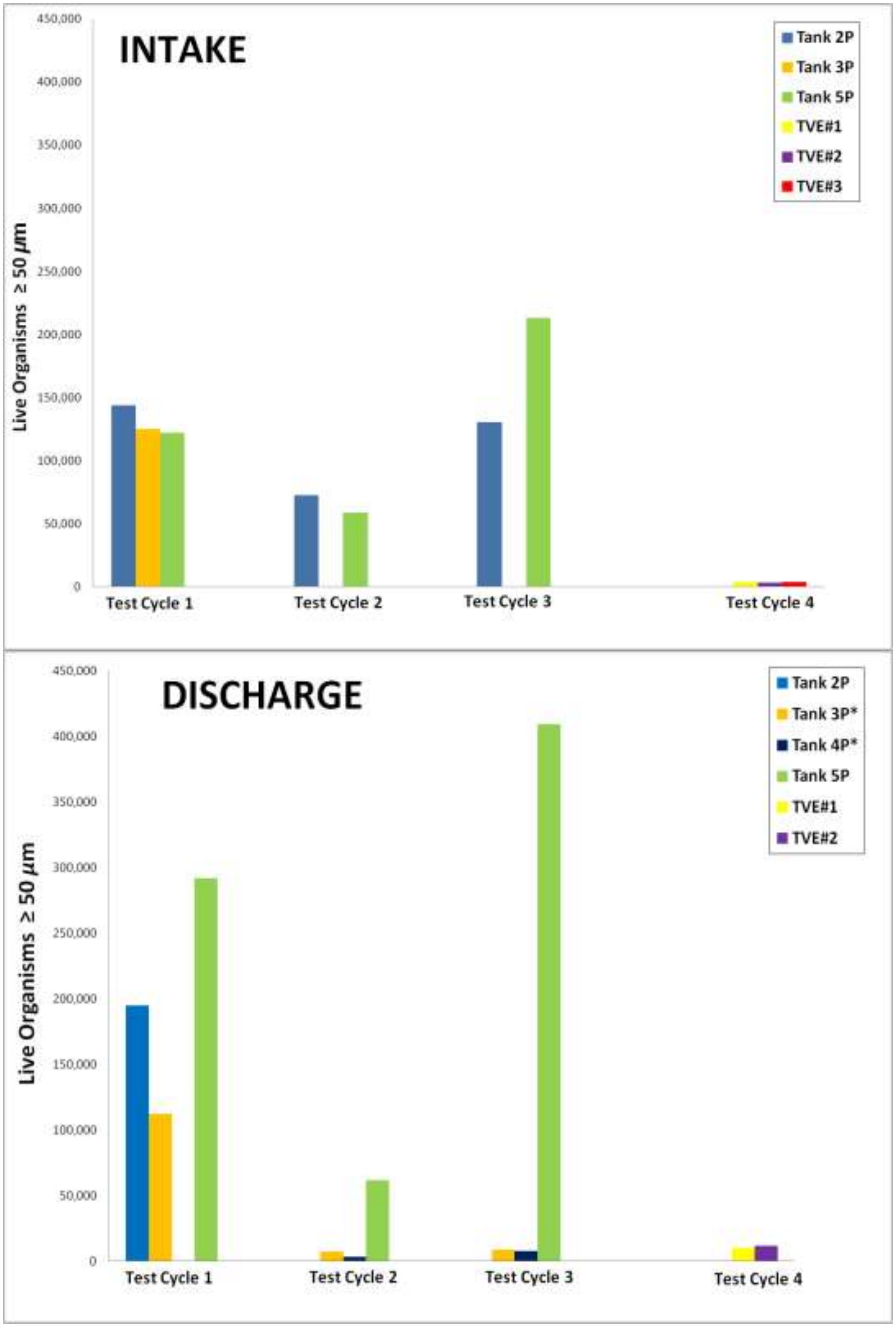



Figure 33. Test Cycle 1-4 intake and discharge concentrations of live organisms ≥ 50 µm. *Tanks 3P and 4P were treated during Test Cycles 2 and 3.

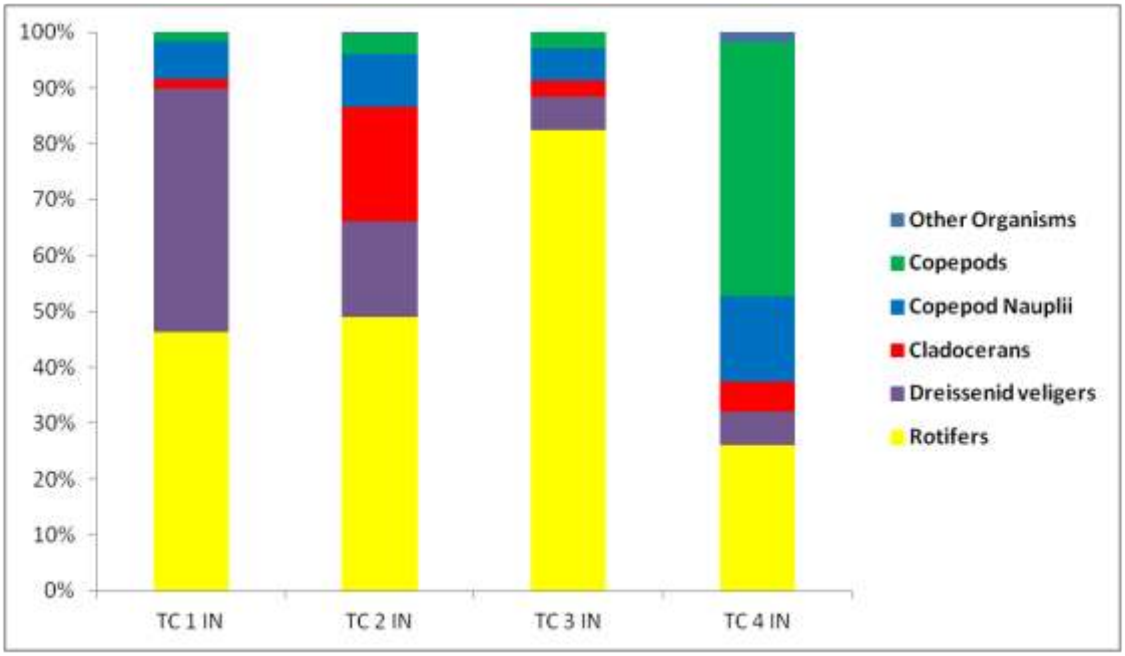



Figure 34. Test Cycle 1-4 intake composition of live organisms ≥ 50 µm.

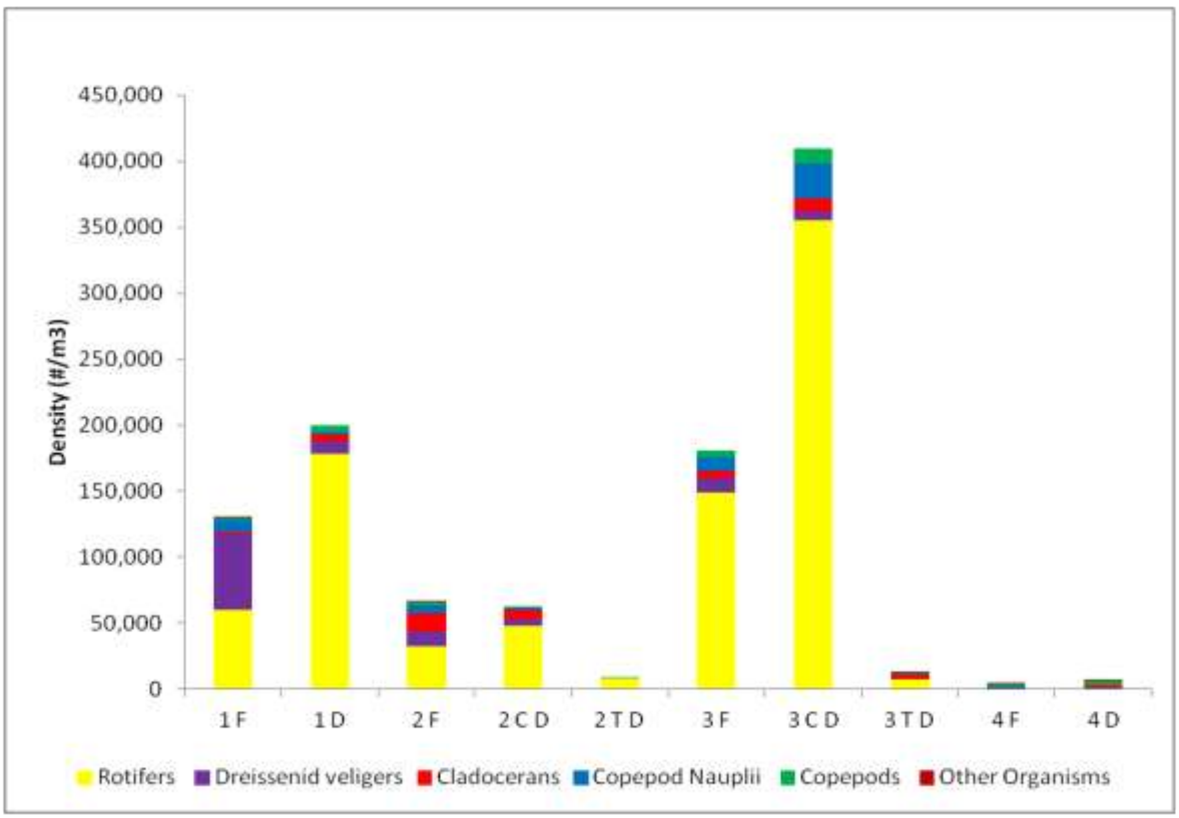



Figure 35. Test Cycle 1-4 intake and discharge density and composition of live organisms ≥ 50 µm.

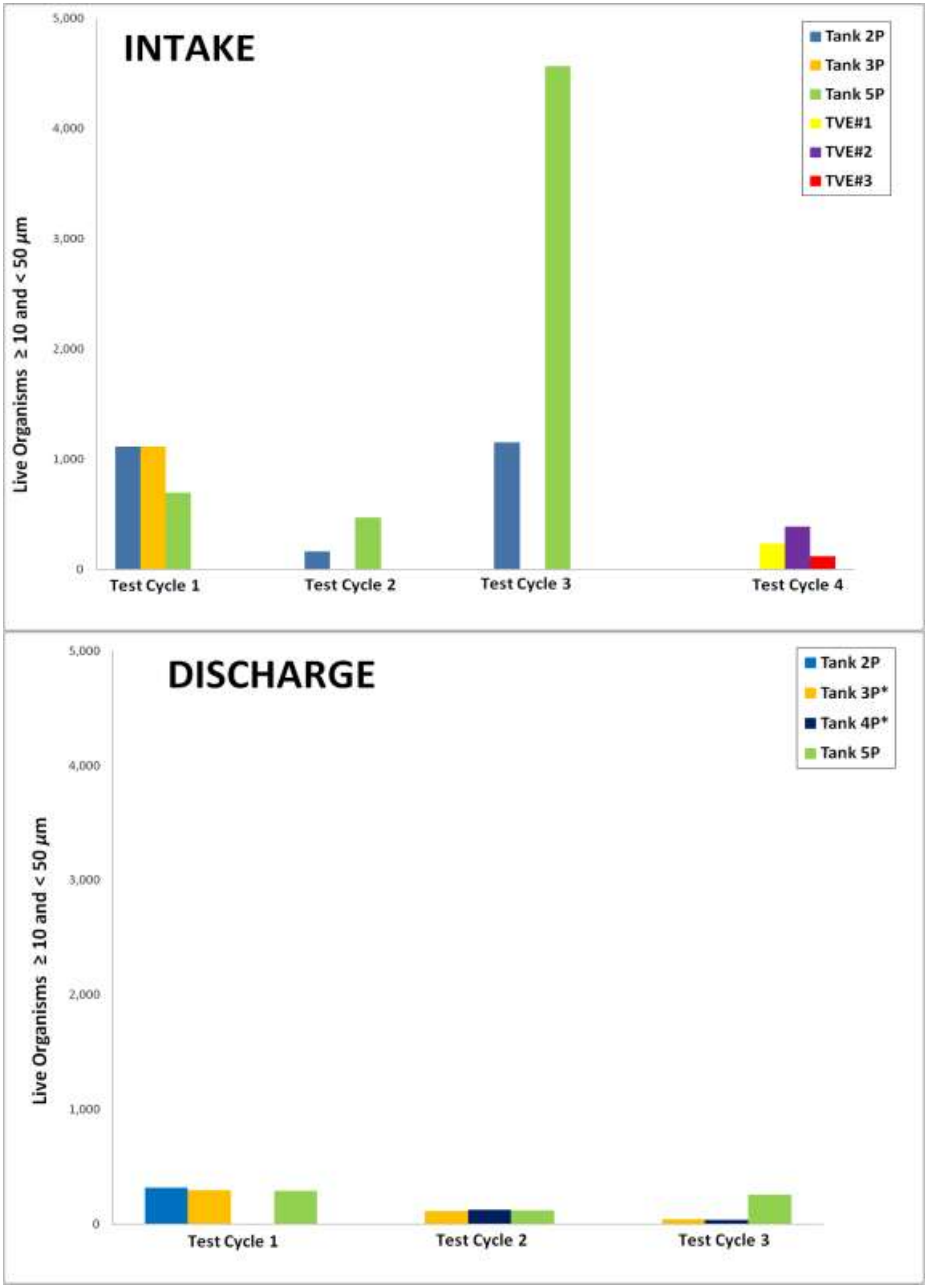



Figure 36. Test Cycle 1-4 intake and discharge concentrations of live organisms ≥ 10 µm and < 50 µm. *Tanks 3P and 4P were treated during Test Cycles 2 and 3.

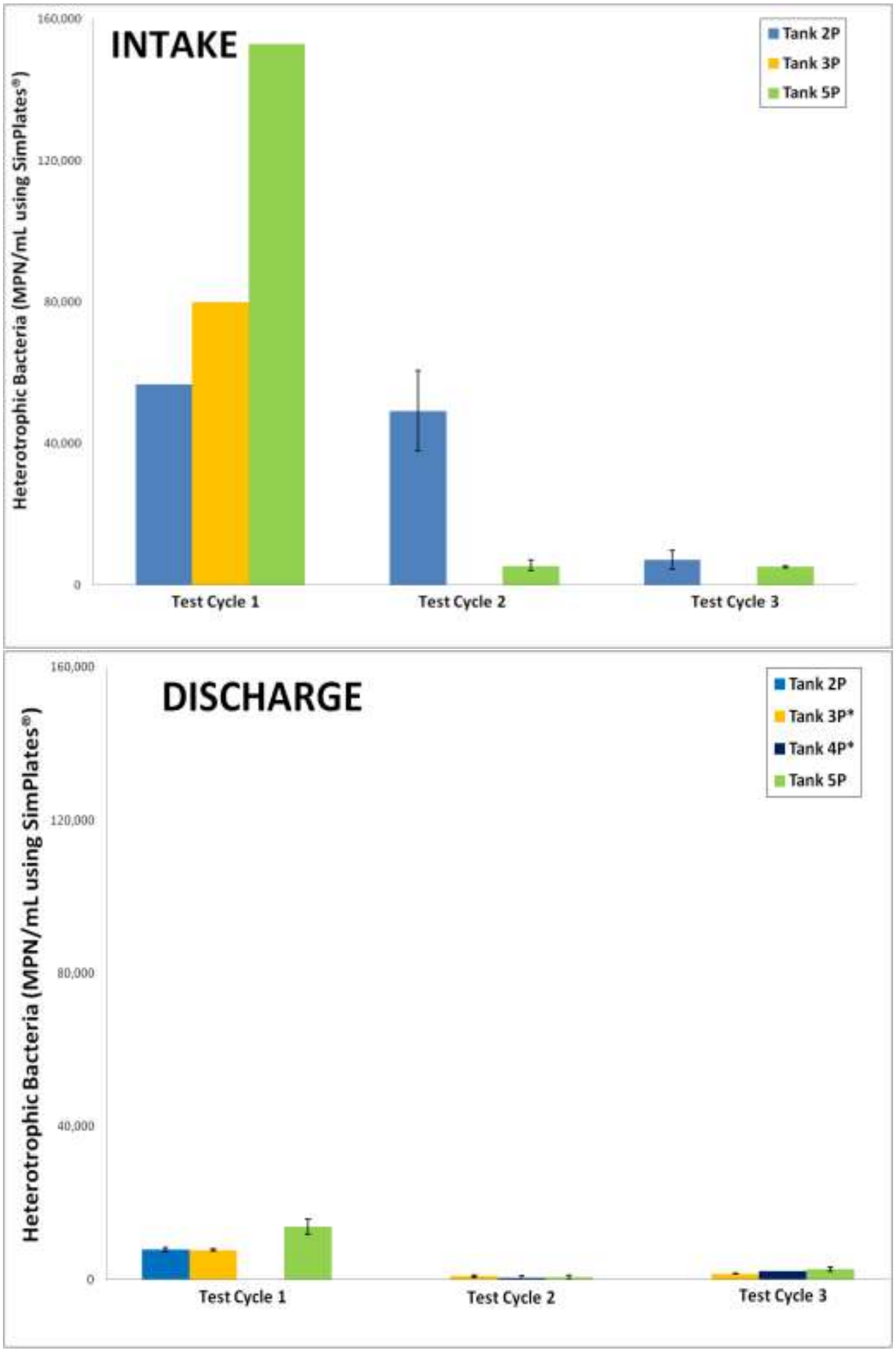



Figure 37. Test Cycle 1-4 intake and discharge concentrations of total heterotrophic bacteria measured using the SimPlate® Method of Analysis.*Tanks 3P and 4P were treated during Test Cycles 2 and 3.

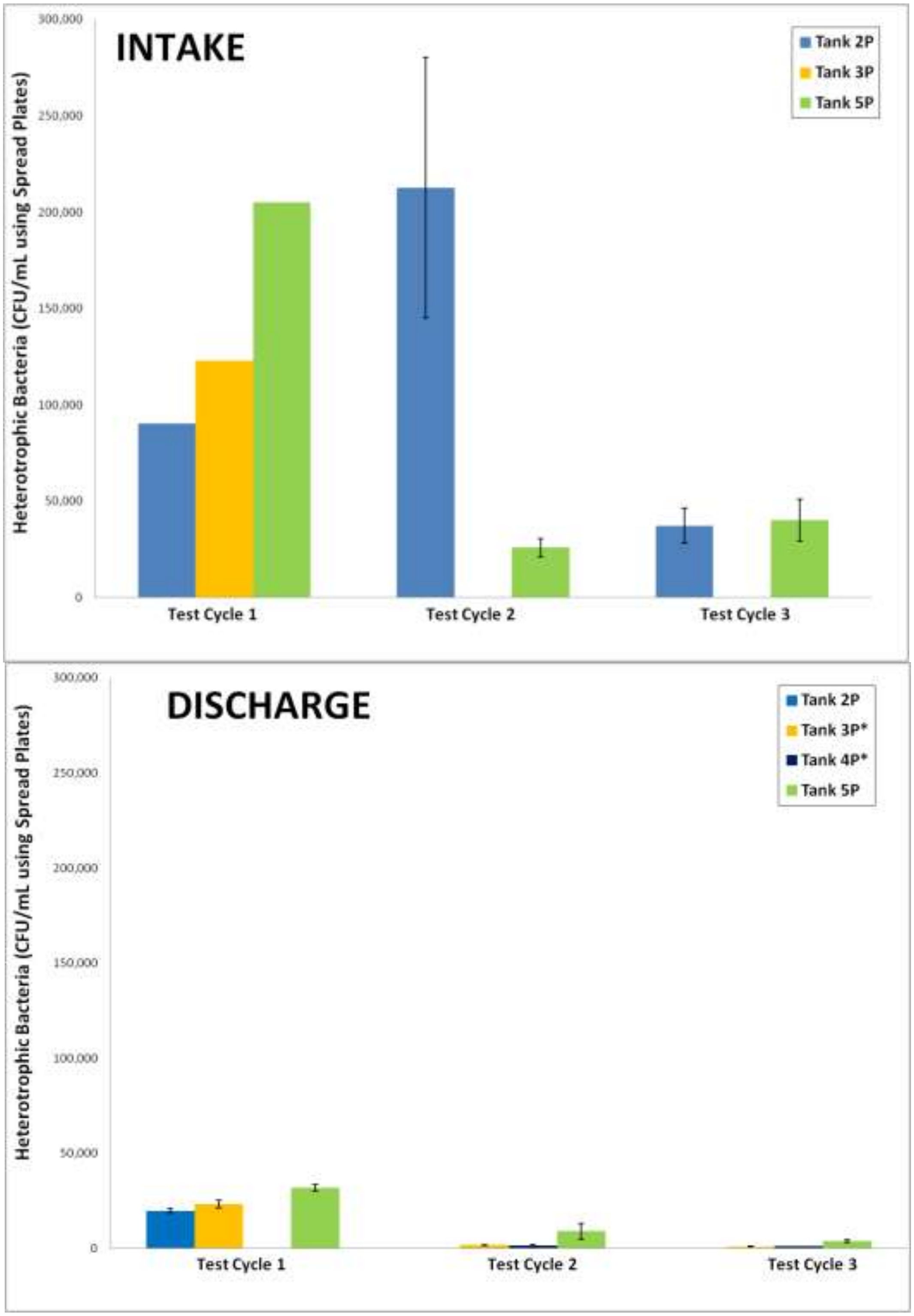



Figure 38. Test Cycle 1-4 intake and discharge concentrations of total heterotrophic bacteria measured using the spread plate method of analysis. *Tanks 3P and 4P were treated during Test Cycles 2 and 3.

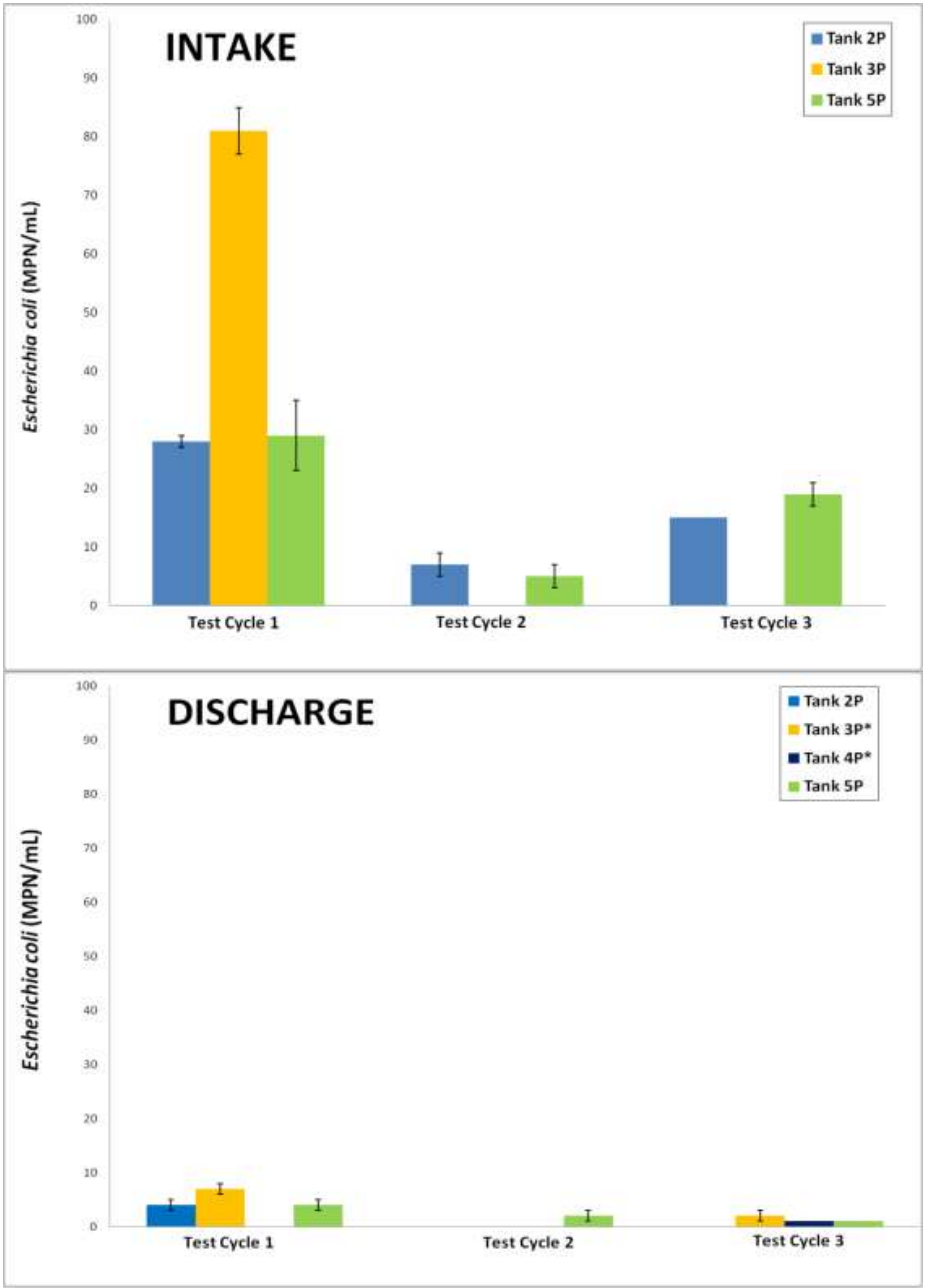



Figure 39. Test Cycle 1-4 intake and discharge concentrations of *Escherichia coli*. *Tanks 3P and 4P were treated during Test Cycles 2 and 3.

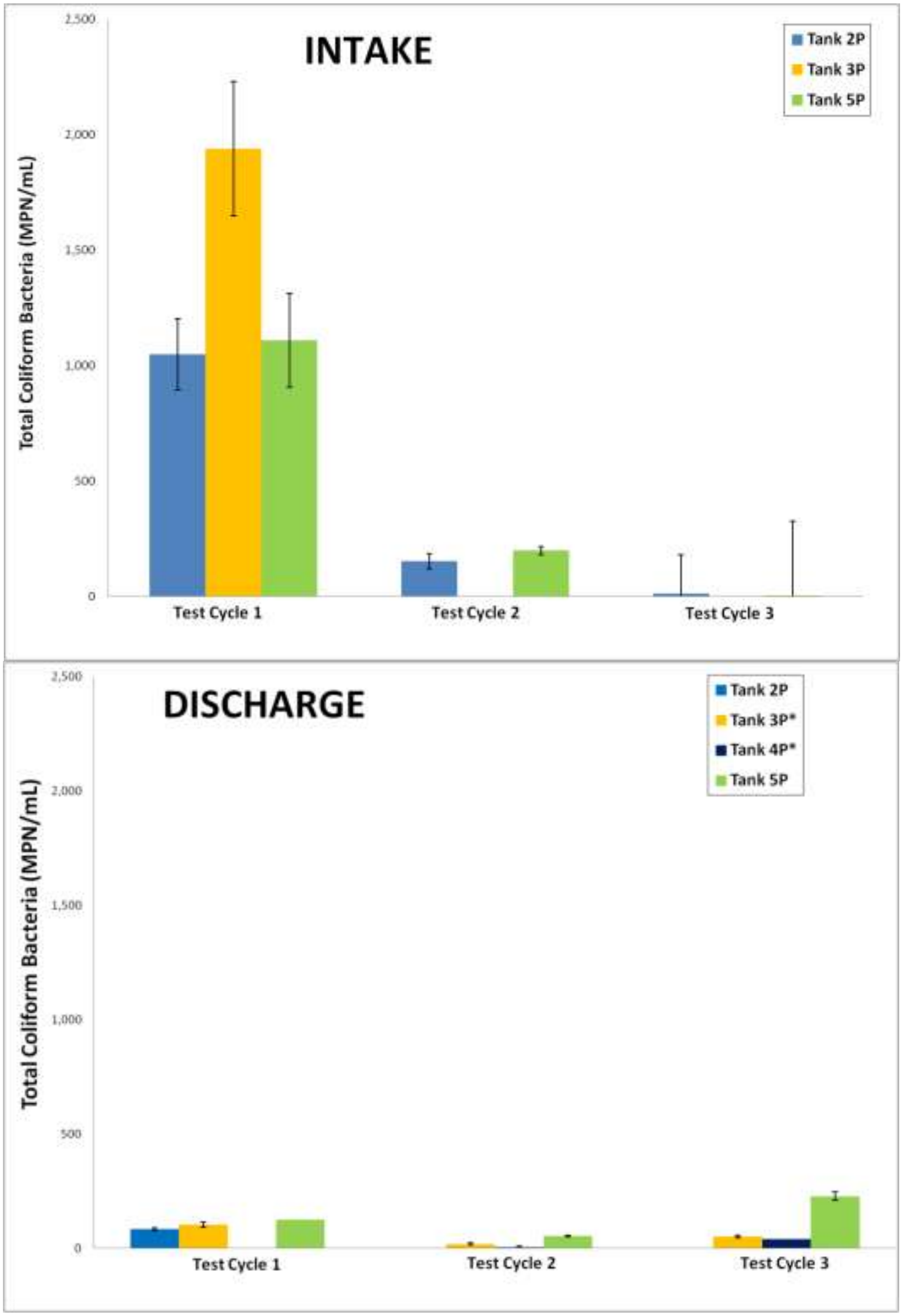



Figure 40. Test Cycle 1-4 intake and discharge concentrations of total coliform bacteria. *Tanks 3P and 4P were treated during Test Cycles 2 and 3.

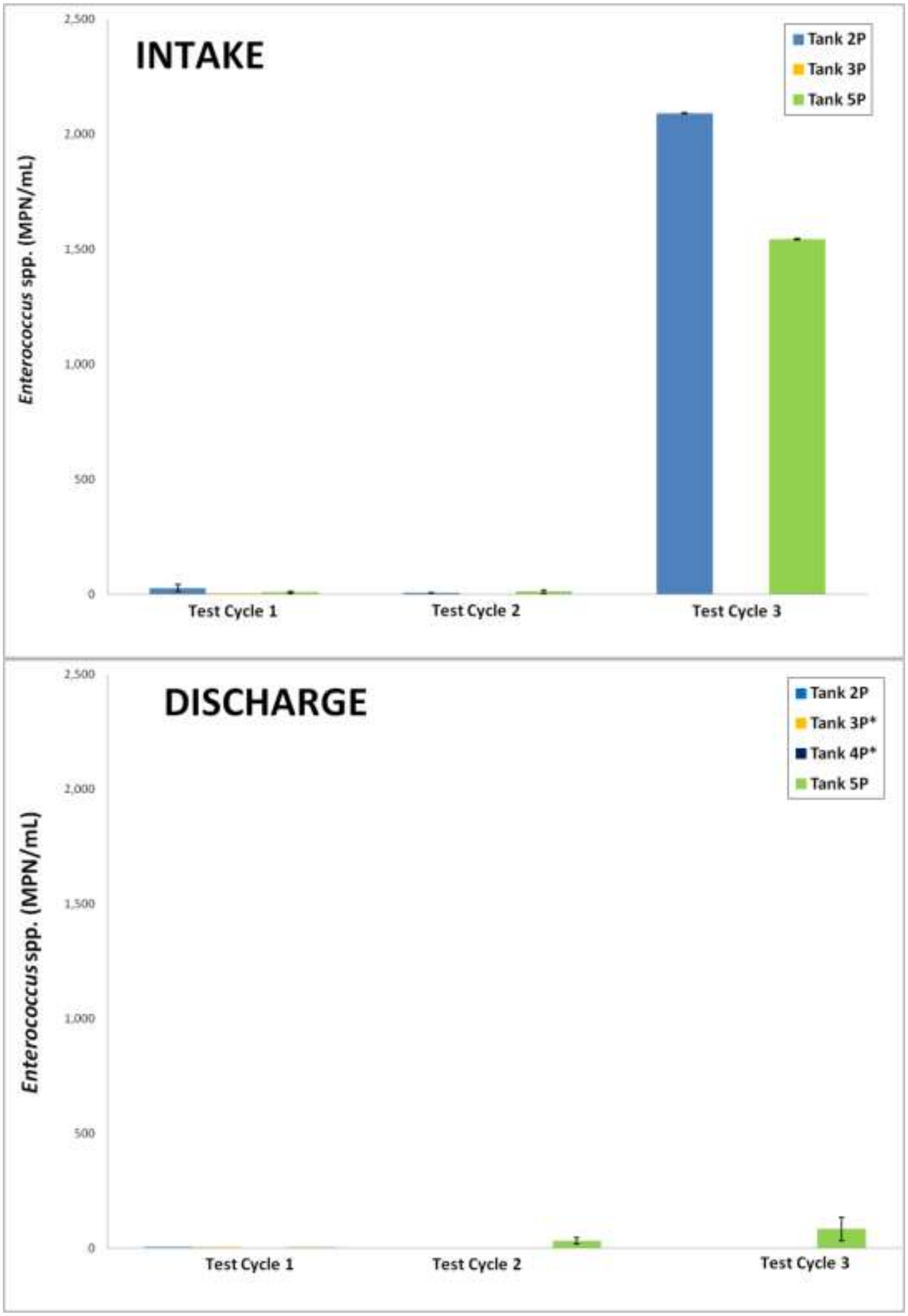



Figure 41. Test Cycle 1-4 intake and discharge concentrations of enterococcus spp. *Tanks 3P and 4P were treated during Test Cycles 2 and 3.

Table 16. Density of organisms (Average ± Standard Error) in intake samples (Test Cycles 1–4). Values marked with an asterisk (*) are outside the valid range for that parameter.

| Test Cycle | Parameter | Unit | Challenge Target Valid Range | Tank 5P | Tank 2P | Tank 3P |
|---|---|---|---|---|---|---|
| 1 | Organisms ≥ 50 $\mu$m[20] | Live #/m$^3$ | ≥ 10,000/m$^3$ | 122,000 | 144,000 | 125,000 |
| | Organisms ≥ 10 and < 50 $\mu$m | Live cells/mL | ≥ 500/mL | 694 | 1,114 | 1,112 |
| | Organisms < 10 $\mu$m -Culturable, Aerobic Heterotrophic Bacteria | Most probable number (MPN)/mL (using SimPlates) | ≥ 500/mL | 153,000 | 56,700 | 80,000 |
| | | Colony forming unit (CFU)/mL (using Spread Plates) | ≥ 500/mL | 205,000 | 90,200 | 123,000 |
| | Total coliform bacteria | MPN/100 mL | No requirement | 1,110 ± 203 | 1,050 ± 154 | 1,940 ± 291 |
| | *Escherichia coli* | MPN/100 mL | No requirement | 29 ± 6 | 28 ± 1 | 81 ± 4 |
| | *Enterococcus* spp. | MPN/100 mL | No requirement | 9 ± 4 | 27 ± 16 | < 1 |
| **Test Cycle** | **Parameter** | **Unit** | **Challenge Target Valid Range** | **Tank 5P** | **Tank 2P** | |
| 2 | Organisms ≥ 50 $\mu$m[21] | Live #/m$^3$ | ≥ 10,000/m$^3$ | 58,600 | 73,700 | |
| | Organisms ≥ 10 and < 50 $\mu$m | Live cells/mL | ≥ 500/mL | 472* | 162* | |
| | Organisms < 10 $\mu$m -Culturable, Aerobic Heterotrophic Bacteria | MPN/mL (using SimPlates) | ≥ 500/mL | 5,400 ± 1,778 | 49,267 ± 11,332 | |
| | | CFU/mL (using Spread Plates) | ≥ 500/mL | 25,778 ± 4,857 | 130,200 ± 67,529 | |
| | Total coliform bacteria | MPN/100 mL | No requirement | 199 ± 18 | 152 ± 33 | |
| | *Escherichia coli* | MPN/100 mL | No requirement | 5 ± 2 | 7 ± 2 | |
| | *Enterococcus* spp. | MPN/100 mL | No requirement | 11 ± 8 | 6 ± 1 | |

[20] Based on assumption that p3SFS flow meter was accurately recording flow rates.
[21] As above.

Table 16. Density of organisms (Average ± Standard Error) in intake samples (Test Cycles 1–4). Values marked with an asterisk (*) are outside the valid range for that parameter (Continued).

| Test Cycle | Parameter | Unit | Challenge Target Valid Range | Tank 5P | Tank 2P | |
|---|---|---|---|---|---|---|
| 3 | Organisms ≥ 50 µm[22] | Live #/$m^3$ | ≥ 10,000/$m^3$ | 220,800 | 123,700 | |
| | Organisms ≥ 10 and < 50 µm | Live cells/mL | ≥ 500/mL | 4,569 | 1,150 | |
| | Organisms < 10 µm -Culturable, Aerobic Heterotrophic Bacteria | MPN/mL (using SimPlates®) | ≥ 500/mL | 7,100 ± 300 | 5,125 ± 2,653 | |
| | | CFU/mL (using Spread Plates) | ≥ 500/mL | 40,111 ± 10,864 | 37,222 ± 8,882 | |
| | Total coliform bacteria | MPN/100 mL | No requirement | 1,544 ± 324 | 2,092 ± 167 | |
| | *Escherichia coli* | MPN/100 mL | No requirement | 19 ± 2 | 15 ± 0 | |
| | *Enterococcus* spp. | MPN/100 mL | No requirement | 3 ± 2 | 12 ± 2 | |
| **Test Cycle** | **Parameter** | **Unit** | **Challenge Target Valid Range** | **Tank Volume Equivalent #1** | **Tank Volume Equivalent #2** | **Tank Volume Equivalent #3** |
| 4 | Organisms ≥ 50 µm[23] | Live #/$m^3$ | ≥ 1,000/$m^3$ | 3,900 | 3,300 | 3,900 |
| | Organisms ≥ 10 and < 50 µm | Live cells/mL | ≥ 500/mL | 233* | 390* | 118* |
| | Organisms < 10 µm - Culturable, Aerobic Heterotrophic Bacteria | MPN/mL (using SimPlates) | ≥ 500/mL | No samples collected due to truncated sampling plan. | | |
| | | CFU/mL (using Spread Plates) | ≥ 500/mL | | | |
| | Organisms < 10 µm – Total coliform bacteria | MPN/100 mL | No requirement | | | |
| | Organisms < 10 µm – *Escherichia coli* | MPN/100 mL | No requirement | | | |
| | Organisms < 10 µm – *Enterococcus* spp. | MPN/100 mL | No requirement | | | |

[22] As above.
[23] Based on assumption that p3SFS flow meter was accurately recording flow rates.

Table 17. Density of organisms (Average ± Standard Error) in discharge samples (Test Cycles 1–4).

| Test Cycle | Parameter | Unit | Tank 2P | Tank 3P | Tank 5P |
|---|---|---|---|---|---|
| 1 | Organisms ≥ 50 $\mu$m[24] | Live #/m$^3$ | 195,000 | 112,000 | 292,000 |
| | Organisms ≥ 10 and < 50 $\mu$m | Live cells/mL | 315 | 298 | 288 |
| | Organisms < 10 $\mu$m -Culturable, Aerobic Heterotrophic Bacteria | Most probable number (MPN)/mL (using SimPlates®) | 7,830 ± 590 | 7,700 ± 300 | 13,800 ± 1,970 |
| | | Colony forming unit (CFU)/mL (using Spread Plates) | 19,700 ± 1,090 | 23,200 ± 1,930 | 31,900 ± 1,750 |
| | Total coliform bacteria | MPN/100 mL | 83 ± 7 | 104 ± 11 | 125 ± 1 |
| | *Escherichia coli* | MPN/100 mL | 4 ± 1 | 7 ± 1 | 4 ± 1 |
| | *Enterococcus* spp. | MPN/100 mL | 3 ± 1 | 4 ± 1 | 3 ± 0 |
| Test Cycle | Parameter | Unit | Tank 3P (Treatment) | Tank 4P (Treatment) | Tank 5P |
| 2 | Organisms ≥ 50 $\mu$m[25] | Live #/m$^3$ | 7,100 | 3,400 | 61,900 |
| | Organisms ≥ 10 and < 50 $\mu$m | Live cells/mL | 116 | 125 | 121 |
| | Organisms < 10 $\mu$m - Culturable, Aerobic Heterotrophic Bacteria | MPN/mL (using SimPlates) | 900 ± 231 | 490 ± 101 | 667 ± 503 |
| | | CFU/mL (using Spread Plates) | 1,691 ± 147 | 1,557 ±300 | 8,911 ± 4,248 |
| | Total coliform bacteria | MPN/100 mL | 18 ± 4 | 5 ± 2 | 54 ± 3 |
| | *Escherichia coli* | MPN/100 mL | < MDL | < MDL | 2 ± 1 |
| | *Enterococcus* spp. | MPN/100 mL | < MDL | < MDL | 31 ± 14 |

[24] Based on assumption that p3SFS flow meter was accurately recording flow rates.
[25] As above.

Table 17. Density of organisms (Average ± Standard Error) in discharge samples (Test Cycles 1–4) (Continued).

| Test Cycle | Parameter | Unit | Tank 3P (Treatment) | Tank 4P (Treatment) | Tank 5P |
|---|---|---|---|---|---|
| 3 | Organisms ≥ 50 µm[26] | Live #/$m^3$ | 8,900 | 7,600 | 409,000 |
| | Organisms ≥ 10 and < 50 µm | Live cells/mL | 45 | 36 | 255 |
| | Organisms < 10 µm - Culturable, Aerobic Heterotrophic Bacteria | MPN/mL (using SimPlates) | 1,567 ± 67 | 2,175 ± 530 | 2,733 ± 463 |
| | | CFU/mL (using Spread Plates) | 935 ± 129 | 1,056 ± 137 | 3,868 ± 632 |
| | Total coliform bacteria | MPN/100 mL | 50 ± 6 | 40 ± 4 | 228 ± 17 |
| | *Escherichia coli* | MPN/100 mL | 2 ± 1 | 1± 0 | <MDL |
| | *Enterococcus* spp. | MPN/100 mL | < MDL | < MDL | 83 ± 51 |
| **Test Cycle** | **Parameter** | **Unit** | **Tank Volume Equivalent #1** | **Tank Volume Equivalent #2** | **Tank Volume Equivalent #3** |
| 4 | Organisms ≥ 50 µm[27] | Live #/$m^3$ | 10,100 | 11,800 | Aborted due to unexpected change in ship ballast operations |
| | Organisms ≥ 10 and < 50 µm | Live cells/mL | No samples collected due to drip-sampler malfunction. | | |
| | Organisms < 10 µm - Culturable, Aerobic Heterotrophic Bacteria | MPN/mL (using SimPlates) | No samples collected due to truncated sampling plan. | | |
| | | CFU/mL (using Spread Plates) | | | |
| | Total coliform bacteria | MPN/100 mL | | | |
| | *Escherichia coli* | MPN/100 mL | | | |
| | *Enterococcus* spp. | MPN/100 mL | | | |

[26] As above.
[27] Based on assumption that p3SFS flow meter was accurately recording flow rates.

## 3.4 Characterization of Test Validity Based on Challenge Conditions

According to the ETV DSP, for a TC to be valid, specific physical/chemical and biological challenge water conditions must be met with minimum target conditions detailed in Tables 5-1 and 5-2 of the protocol (USEPA, 2012). The ETV DSP states that while challenge water conditions for biological size fractions must meet the specified target values in four of five valid biological treatment efficacy tests, the fifth test must contain > 75 % of the specified challenge concentrations (USEPA, 2012). Failure to meet physical/chemical targets will not invalidate a TC, unless the challenge water conditions are less than half of the specified targets (USEPA, 2012). As such, GSI target biological and physical/chemical challenge water requirements for TCs 1 through 4 were consistent with the ETV DSP. However, it should be noted that the ETV DSP requirement for cells ≥ 10 and < 50 $\mu$m in <u>minimum</u> dimension is generally not met in GSI analyses which measure and report cells ≥ 10 $\mu$m in <u>any</u> dimension, consistent with GSI's USEPA ETV-audited and accepted SOPs. Target vs. actual measurements for TCs 1-4 across key parameters are detailed in Tables 12, 14 and 16.

TC1 parameters exceeded target levels except in the area of water chemistry; neither TSS nor POM minimum challenge water targets were met (Table 12). Only one of the TSS samples, 7.6 mg/L measured in tank 5P intake, was more than 50 % of the ≥ 12 mg/L target (Table 12). These low TSS and POM values were likely the result of new ship practices in which the IH ballasts using a high sea chest to reduce sediment and organism entrainment in ballast.

For TC2, the target POM level of ≥ 2 mg/L was not met, but intake concentrations were more than 50 % of the target (Table 12). The biological challenge water requirement for the ≥ 10 and < 50 $\mu$m size class was borderline even using the GSI method of counting, and would require a decision from the VO to determine validity; tank 5P had 470 cell/mL and tank 2P had 160 cells/mL (Table 16). Samples from tank 5P did contain > 75 % of the specified challenge concentration for organisms ≥ 10 and < 50 $\mu$m, however samples from tank 2P did not (Table 16).

All TC3 physical/chemical and biological challenge water targets were met except for TSS concentrations in tank 2P (Tables 12, 14 and 16). Failure to meet the TSS target did not invalidate the test since the measured TSS value of 9.3 mg/L was still more than half of the minimum target value (Table 12).

All TC4 physical/chemical and biological challenge water targets were exceeded except for presumed live densities of organisms ≥ 10 $\mu$m and < 50 $\mu$m which were below the target density of 500/mL (Table 12, 14 and 16). Concentrations in the TVEs ranged from 118/mL to 390/mL such that only one of the samples was greater than 75 % of the specified requirement (Table 16).

## 3.5 Biological Performance (BWMS) Efficacy

Consistent with the ETV DSP, GSI analyzed treatment discharge data from TCs 2 and 3, the only TCs where the BWMS was active, against the USCG's Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters (USCG, 2012) to determine the biological treatment efficacy of the prototype NaOH BWMS. For organisms ≥ 50 µm in minimum dimension, the USCG standard's maximum concentration allowable in treatment discharge is less than 10 live organisms per m3 (USCG, 2012; Table

18). Live concentrations of organisms ≥ 50 $\mu$m measured in treatment discharge from tanks 3P and 4P during TCs 2 and 3 were three orders of magnitude above this level[28] (Table 18). However, the faulty data from the p3SFS flow meter, discovered after the end of the testing, introduced error into the measured ballast discharge concentrations.

For organisms ≥ 10 $\mu$m and < 50 $\mu$m in minimum dimension, the whole water sampling system assured representative values for the volumes sampled. The USCG standard's maximum concentration allowable in treatment discharge is less than 10 live organisms per mL (USCG, 2012; Table 18). The BWMS delivered live concentrations of organisms ≥ 10 $\mu$m and < 50 $\mu$m in treatment discharge from tanks 3P and 4P during TCs 2 and 3 that were two orders of magnitude above this regulatory benchmark (Table 18). Similarly, values reported in Table 18 for organisms < 10 $\mu$m in minimum dimension are likely representative. The USCG standard's maximum concentration allowable in treatment discharge is < 250 CFU/100 mL for *E. coli* and < 100 CFU/100 mL for *Enterococcus* spp. (Table 18). As noted above, intake concentrations for both species were generally low, and below the discharge limit for *E. coli* and *Enterococcus spp.* in TCs 1-3 (Table 18). Hence, concentrations of these two organisms in treatment discharge from tanks 3P and 4P during TCs 2 and 3 were well below these levels (Table 18).

Table 18. Biological concentrations in treated discharge by size class from Test Cycles 2 and 3 compared to maximum treated discharge concentrations specified in the U.S. Coast Guard's *Standards for Living Organisms in Ships' Ballast Water Discharged in U.S. Waters* (USCG, 2012). MDL = Method Detection Limit.

| Organism Size Class | USCG Standard: Maximum Concentration in Treated Discharge | Test Cycle 2 | | Test Cycle 3 | |
|---|---|---|---|---|---|
| | | Tank 3P | Tank 4P | Tank 3P | Tank 4P |
| Organisms ≥ 50 $\mu$m in minimum dimension | < 10 live organisms per $m^3$ | 7,100[29] | 3,400[30] | 8,800[31] | 7,600[32] |
| Organisms ≥ 10 $\mu$m and < 50 $\mu$m in minimum dimension | < 10 live organisms per mL | 116 | 125 | 45 | 36 |
| Organisms < 10 $\mu$m in minimum dimension | < 250 colony forming unit (CFU)/100 mL of *Escherichia coli* | < MDL | < MDL | 2 ± 1 | 11± 0 |
| | < 100 CFU/100 mL of *Enterococcus* | < MDL | < MDL | < MDL | < MDL |

## 3.6 Environmental Acceptability

GSI analyzed treated discharge from TCs 2 and TC3, the only TCs where the BWMS was active, with respect to environmental acceptability of the BWMS-treated ballast discharges. Environmental acceptability was determined by the presence of disinfection byproducts in treatment discharge analyzed by Analytical Laboratory Services (Middletown, Pennsylvania), and WET of treatment discharge versus receiving water controls, i.e., Duluth-Superior Harbor water, relative to three species: the cladoceran *Ceriodaphnia dubia*, the fathead minnow *Pimephales promelas*, and the green alga *Selenastrum capricornutum*. As with the protist and microbial results, the results of these tests were unaffected by p3SFS flow meter/control malfunctions and can be considered reliable.

[28] Based on assumption that p3SFS flow meter was accurately recording flow rates.
[29] As above.
[30] As above.
[31] As above.
[32] As above.

### 3.6.1 Disinfection Byproducts

No trihalomethanes, haloacetic acids, or bromate ions were detected in the TC2 and TC3 discharge samples (Table 19). Measurable concentrations of sodium ion were found in the treatment discharge from tanks 3P and 4P (Table 19). For TC2, the sodium concentration of the treated tanks (3P and 4P) ranged from 159 - 170 µg/L, which was substantially higher than that of the untreated tank (5P) of 10.1 µg/L sodium (Table 19). For TC3, the sodium ion concentration in discharge was higher than TC2, with tank 4P discharge again slightly higher at 343 µg/L (Table 19); tank 3P discharge was 335 µg/L (Table 19). In comparison, untreated tank 5P discharge had a sodium concentration of 17.3 µg/L (Table 19). The higher sodium levels in TC3 treated discharge relative to TC2 coincide with a higher target pH level of 11.7 in TC3 relative to 11.5 in TC2. Chlorate levels were also higher in TC3 compared to TC2 (Table 19). TC2 chlorate concentrations were 590 µg/L in tank 3P discharge and 575 µg/L in tank 4P discharge (Table 19). TC3 chlorate concentrations were 1,470 µg/L in tank 3P discharge and 1,710 µg/L in tank 4P discharge (Table 19). Chlorate concentrations were not detectable in any TC2 or TC3 untreated discharge samples (Table 19).

Table 19. Concentrations of disinfection byproducts measured in Test Cycle 2 and 3 treated and untreated discharge samples. MDL = Method Detection Limit.

| Class | Analyte | Test Cycle 2 | | | Test Cycle 3 | | |
|---|---|---|---|---|---|---|---|
| | | Tank 3P Treatment (µg/L) | Tank 4P Treatment (µg/L) | Tank 5P Untreated (µg/L) | Tank 3P Treatment (µg/L) | Tank 4P Treatment (µg/L) | Tank 5P Untreated (µg/L) |
| Trihalomethanes | Bromodichloromethane ($CHBrCl_2$) | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| | Bromoform ($CHBr_3$) | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| | Chlorodibromomethane ($CHBr_2Cl$) | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| | Chloroform ($CHCl_3$) | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| | Total Trihalomethanes | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 | < 0.5 |
| Haloacetic Acids | Bromochloroacetic acid (BrClCHCOOH) | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 |
| | Dibromoacetic acid ($CHBr_2COOH$) | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 |
| | Dichloroacetic acid ($CHCl_2COOH$) | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 |
| | Monobromoacetic acid ($CH_2BrCOOH$) | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 |
| | Monochloroacetic acid ($CH_2ClCOOH$) | < 2.0 | < 2.0 | < 2.0 | < 2.0 | < 2.0 | < 2.0 |
| | Trichloroacetic acid ($CCl_3COOH$) | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 |
| | Total Haloacetic Acids | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 | < 1.0 |
| Sodium | Sodium (Na) | 159 | 170 | 10.1 | 335 | 343 | 17.3 |
| Others | Bromate ($BrO_3^-$) | < 5.0 | < 5.0 | < 5.0 | < 5.0 | < 5.0 | < 5.0 |
| | Chlorate ($ClO_3^-$) | 590 | 575 | < 20 | 1,470 | 1,710 | < 200 |

### 3.6.2 Whole Effluent Toxicity

#### *3.6.2.1 Cladoceran (Ceriodaphnia dubia) Survival and Reproduction*

Results from TC2 and TC3 WET tests conducted on *C. dubia* are presented in Table 20. There were no significant differences ($p$>0.05) between percent survival of *C. dubia* exposed to filtered Duluth-Superior Harbor water (i.e., the receiving water control), untreated effluent from tank 5P, and treated effluent from tanks 3P and 4P (all dilutions; Table 20).

There were significant differences in *C. dubia* reproduction across sample types. In TC2, reproduction in the 50 % and 100 % tank 3P treatment groups (16.5 ± 2.8 and 6.4 ± 1.9 young per female, respectively) was significantly ($p$<0.05) lower than in the filtered Duluth-Superior Harbor water group, which had an average number of 27.8 ± 1.8 young per female in three broods (Table 20). There was a similar significant ($p$<0.05) difference in the number of young per female between the 12.5 %, 25 %, 50 %, and 100 % treatment groups from tank 4P (21.0 ± 2.4, 18.3 ± 3.0, 13.8 ± 2.4 and 8.6 ± 1.4 young per female, respectively) and the filtered Duluth-Superior Harbor water group (Table 20). Reproduction in effluent from the 100 % tank 3P, and 50 % and 100 % tank 4P groups was significantly ($p$<0.05) lower than in the untreated whole effluent from tank 5P group (Table 20). In TC3, there was significantly ($p$<0.05) less reproduction in the 100 % exposures from tanks 3P and 4P (7.7 ± 1.0 and 3.6 ± 1.0 young per female, respectively) relative to the receiving water control (26.9 ± 3.4 young per female; Table 20). *C. dubia* reproduction in the 100 % effluent from the two treatment tanks was significantly ($p$<0.05) less than in the 100 % tank 5P effluent (26.9 ± 3.3 young per female; Table 20).

Results for temperature and pH, measured daily, and hardness and alkalinity, measured on test termination day (Day 5), for TC2 and TC3 are presented in Table 21. Temperature ranged from 22.8 ºC to 25.1 ºC across all treatment groups and TCs, while pH ranged from a minimum of 7.78 in the performance control, i.e., HRW, to a maximum of 8.91 in TC2's 100 % effluent from tank 4P (Table 21). Hardness measured highest in the performance control, followed by the untreated effluent from tank 5P, and lowest in the 100 % treated effluent from tanks 3P and 4P (Table 21). Conversely, alkalinity measured highest in the 100 % treated effluent from tanks 3P and 4P, and lowest in the receiving water control (Table 21).

Table 20. Percent survival (Average ± Standard Error; n = 10) and total number of offspring per female (Average ± Standard Error; n = 10) in a three-brood *Ceriodaphnia dubia* whole effluent toxicity test after 5 days exposure to treated and untreated ballast discharge collected during Test Cycles 2 and 3.

| Treatment Group | Exposure Solution | Test Cycle 2 | | Test Cycle 3 | |
|---|---|---|---|---|---|
| | | Percent Survival | Total Number of Young per Female | Percent Survival | Total Number of Young per Female |
| Performance Control[A] | N/A | 80 ± 13 | 10.3 ± 2.0 | 100 ± 0 | 24.6 ± 1.4 |
| Receiving Water Control | Filtered Duluth-Superior Harbor Water | 100 ± 0 | 27.8 ± 1.8 | 90 ± 10 | 26.9 ± 3.3 |
| Tank 5P (Untreated) | 100 % | 100 ± 0 | 25.4 ± 2.5 | 100 ± 0 | 29.6 ± 2.1 |
| Tank 3P (Treated) | 6.25 % | 100 ± 0 | 21.8 ± 4.0 | 100 ± 0 | 28.7 ± 1.3 |
| | 12.5 % | 90 ± 10 | 28.7 ± 2.3 | 90 ± 10 | 25.7 ± 1.4 |
| | 25 % | 100 ± 0 | 24.4 ± 2.2 | 100 ± 0 | 20.9 ± 2.5 |
| | 50 % | 100 ± 0 | 16.5 ± 2.8^ | 100 ± 0 | 21.2 ± 2.3 |
| | 100 % | 100 ± 0 | 6.4 ± 1.9^* | 90 ± 10 | 7.7 ± 1.0^* |
| Tank 4P (Treated) | 6.25 % | 90 ± 10 | 26.0 ± 1.6 | 100 ± 0 | 24.0 ± 1.6 |
| | 12.5 % | 100 ± 0 | 21.0 ± 2.4^ | 90 ± 10 | 26.0 ± 1.1 |
| | 25 % | 90 ± 10 | 18.3 ± 3.0^ | 100 ± 0 | 24.8 ± 1.3 |
| | 50 % | 90 ± 10 | 13.8 ± 2.4^* | 100 ± 0 | 19.0 ± 1.4^* |
| | 100 % | 100 ± 0 | 8.6 ± 1.4^* | 80 ± 13 | 3.6 ± 1.0^* |

[A] Hard reconstituted water (U.S. Environmental Protection Agency Office of Water, 2002)

^ The difference in average number of young per female are statistically ($p$<0.05) different from the receiving water control.

***** The difference in average number of young per female are statistically ($p$<0.05) different from the untreated 100 % whole effluent from tank 5P.

Table 21. Average (Minimum, Maximum) water chemistry parameters measured in exposure solutions during the *Ceriodaphnia dubia* whole effluent toxicity tests for Test Cycles 2 and 3.

| Treatment Group | Exposure Solution | Test Cycle 2 | | | | Test Cycle 3 | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | Temp. (°C) | pH | Hardness (mg/L $CaCO_3$) | Alkalinity (mg/L $CaCO_3$) | Temp. (°C) | pH | Hardness (mg/L $CaCO_3$) | Alkalinity (mg/L $CaCO_3$) |
| Performance Control[A] | N/A | 23.9 (23.1, 24.3) | 8.20 (8.13, 8.25) | 178.0 | 124.0 | 24.1 (23.1, 24.8) | 8.03 (7.78, 8.20) | 172.4 | 122.0 |
| Receiving Water Control | Filtered Duluth-Superior Harbor water | 24.1 (23.6, 24.7) | 8.07 (7.92, 8.17) | 77.6 | 72.0 | 24.2 (23.4, 24.5) | 7.94 (7.88, 8.02) | 70.4 | 62.4 |
| Tank 5P (Untreated) | 100 % | 24.3 (24.0, 24.6) | 8.25 (8.18, 8.30) | 144.4 | 117.2 | 24.3 (23.6, 24.7) | 8.34 (8.29, 8.40) | 167.6 | 153.2 |
| Tank 3P (Treated) | 6.25 % | 24.1 (23.9, 24.3) | 8.25 (8.19, 8.32) | 74.8 | 94.4 | 24.7 (24.5, 25.1) | 8.22 (8.10, 8.32) | 67.2 | 102.4 |
| | 12.5 % | 24.3 (24.0, 24.4) | 8.29 (8.21, 8.34) | 68.4 | 108.8 | 24.6 (24.4, 25.1) | 8.37 (8.32, 8.41) | 64.4 | 147.2 |
| | 25 % | 24.1 (23.8, 24.4) | 8.37 (8.27, 8.46) | 63.6 | 146.8 | 24.6 (24.0, 25.1) | 8.58 (8.54, 8.61) | 62.0 | 235.6 |
| | 50 % | 24.2 (23.9, 24.5) | 8.61 (8.58, 8.65) | 48.8 | 221.6 | 24.7 (24.4, 25.1) | 8.80 (8.72, 8.85) | 49.2 | 404.8 |
| | 100 % | 23.9 (23.4, 24.3) | 8.82 (8.78, 8.85) | 22.8 | 377.2 | 24.6 (24.1, 25.0) | 8.97 (8.95, 9.00) | 30.4 | 747.6 |
| Tank 4P (Treated) | 6.25 % | 23.7 (23.1, 24.1) | 8.20 (8.04, 8.33) | 78.8 | 90.8 | 24.2 (23.7, 24.7) | 8.27 (8.16, 8.43) | 66.0 | 107.6 |
| | 12.5 % | 24.4 (24.1, 24.6) | 8.28 (8.19, 8.35) | 70.4 | 110.8 | 24.5 (24.3, 24.7) | 8.39 (8.36, 8.44) | 64.4 | 148.8 |
| | 25 % | 24.4 (23.6, 25.0) | 8.41 (8.34, 8.52) | 60.8 | 152.8 | 24.4 (24.2, 24.6) | 8.54 (8.51, 8.57) | 58.0 | 238.0 |
| | 50 % | 24.1 (23.7, 24.8) | 8.64 (8.61, 8.70) | 50.8 | 226.8 | 24.3 (24.1, 24.7) | 8.82 (8.77, 8.86) | 48.0 | 416.8 |
| | 100 % | 24.1 (23.6, 24.6) | 8.87 (8.82, 8.91) | 20.0 | 388.4 | 24.1 (22.8, 24.6) | 9.01 (8.97, 9.07) | 27.2 | 759.2 |

[A] Hard reconstituted water (U.S. Environmental Protection Agency Office of Water, 2002)

### *3.6.2.2 Fathead Minnow (Pimephales promelas) Survival and Reproduction*

Results from TC2 and TC3 WET tests conducted on *P. promelas* are presented in Table 22. There were no significant differences ($p$>0.05) in survival or growth of *P. promelas* exposed to filtered Duluth-Superior Harbor water, i.e., the receiving water control, and *P. promelas* exposed to effluent from the untreated tank 5P and the treated tanks 3P and 4P (all dilutions; Table 22). *P. promelas* exposed to filtered Duluth-Superior Harbor water, 100 % whole effluent from the untreated tank 5P, and 100 % whole effluent from the treated tanks 3P and 4P all had 95 % or greater survival (Table 22). Mean average weight of fish was similar across all treatment groups and dilutions, ranging 0.41 mg to 0.48 mg (Table 22).

Results for temperature, pH and dissolved oxygen which were measured daily and hardness and alkalinity which were measured on test termination day (Day 7), are presented in Table 23. Temperature ranged from 22.3 ºC to 26.8 ºC across all treatment groups, while pH ranged from a minimum of 7.22 in the TC2 performance control to a maximum of 8.74 in the TC3 100 % effluent from tanks 3P and 4P (Table 23). Dissolved oxygen concentration ranged from a minimum of 3.7 mg/L to 6.7 mg/L (Table 23). Hardness measured highest in the effluent from the untreated tank 5P and lowest in the 100 % effluent from tanks 3P and 4P (Table 23). Conversely, alkalinity measured highest in the 100 % effluent from tanks 3P and 4P, and lowest in the performance control (Table 23).

Table 22. Percent survival (Average ± Standard Error; n = 4) and weight (Average ± Standard Error; n = 4) in a *Pimephales promelas* whole effluent toxicity test after 7 days exposure to treated and untreated ballast discharge collected during Test Cycles 2 and 3.

| Treatment Group | Exposure Solution | Test Cycle 2 | | Test Cycle 3 | |
|---|---|---|---|---|---|
| | | Percent Survival | Weight/Fish (mg) | Percent Survival | Weight/Fish (mg) |
| Performance Control[A] | N/A | 98 ± 1.7 | 0.41 ± 0.01 | 100 ± 0 | 0.41 ± 0.02 |
| Receiving Water Control | Filtered Duluth-Superior Harbor water | 98 ± 1.7 | 0.44 ± 0.01 | 95 ± 0.48 | 0.42 ± 0.02 |
| Tank 5P (Untreated) | 100 % | 98 ± 1.7 | 0.39 ± 0.02 | 97 ± 0.29 | 0.43 ± 0.01 |
| Tank 3P (Treated) | 6.25 % | 97 ± 1.9 | 0.42 ± 0.01 | 100 ± 0 | 0.48 ± 0.01 |
| | 12.5 % | 97 ± 1.9 | 0.43 ± 0.01 | 100 ± 0 | 0.46 ± 0.03 |
| | 25 % | 98 ± 1.7 | 0.42 ± 0.01 | 100 ± 0 | 0.42 ± 0.01 |
| | 50 % | 100 ± 0 | 0.42 ± 0.01 | 100 ± 0 | 0.43 ± 0.01 |
| | 100 % | 98 ± 1.7 | 0.43 ± 0.01 | 100 ± 0 | 0.47 ± 0.01 |
| Tank 4P (Treated) | 6.25 % | 100 ± 0 | 0.47 ± 0.02 | 100 ± 0 | 0.45 ± 0.01 |
| | 12.5 % | 98 ± 1.7 | 0.43 ± 0.03 | 98 ± 0.25 | 0.48 ± 0.02 |
| | 25 % | 97 ± 1.9 | 0.49 ± 0.01 | 100 ± 0 | 0.47 ± 0.02 |
| | 50 % | 98 ± 1.7 | 0.48 ± 0.01 | 98 ± 0.25 | 0.47 ± 0.02 |
| | 100 % | 98 ± 1.7 | 0.49 ± 0.01 | 100 ± 0 | 0.48 ± 0.02 |

[A] Dechlorinated Laboratory Water

Table 23. Average (Minimum, Maximum) water chemistry parameters measured in exposure solutions during the *Pimephales promelas* whole effluent toxicity tests for Test Cycles 2 and 3.

| Treatment Group | Exposure Solution | Test Cycle 2 | | | | | Test Cycle 3 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Temp. (°C) | pH | Dissolved Oxygen (mg/L) | Hardness (mg/L $CaCO_3$) | Alkalinity (mg/L $CaCO_3$) | Temp. (°C) | pH | Dissolved Oxygen (mg/L) | Hardness (mg/L $CaCO_3$) | Alkalinity (mg/L $CaCO_3$) |
| Performance Control[A] | N/A | 24.3 (23.2, 25.6) | 7.34 (7.22, 7.52) | 5.4 (4.5, 6.6) | 48.4 | 50.0 | 24.5 (23.2, 25.8) | 7.47 (7.32, 7.65) | 6.7 (5.7, 7.4) | 48.0 | 50.8 |
| Receiving Water Control | Filtered Duluth-Superior Harbor water | 24.1 (23.4, 25.3) | 7.47 (7.34, 7.61) | 5.4 (4.7, 6.4) | 75.6 | 68.0 | 24.4 (23.6, 25.8) | 7.56 (7.29, 7.73) | 6.4 (4.6, 7.1) | 69.2 | 59.2 |
| Tank 5P (Untreated) | 100 % | 23.8 (23.0, 25.4) | 7.85 (7.74, 8.10) | 5.7 (4.8, 6.7) | 144.4 | 116.0 | 24.4 (24.0, 25.5) | 8.08 (7.95, 8.26) | 6.3 (5.4, 7.0) | 162.4 | 116.4 |
| Tank 3P (Treated) | 6.25 % | 24.2 (22.9, 24.8) | 7.72 (7.58, 7.87) | 5.5 (4.8, 6.1) | 71.6 | 85.6 | 24.3 (22.3, 25.2) | 7.84 (7.65, 7.97) | 6.2 (4.5, 7.5) | 64.0 | 98.4 |
| | 12.5 % | 24.2 (23.2, 25.1) | 7.76 (7.65, 7.91) | 5.1 (4.2, 6.1) | 66.4 | 105.2 | 24.4 (23.3, 25.9) | 8.07 (7.91, 8.16) | 6.3 (4.9, 7.1) | 62.8 | 141.2 |
| | 25 % | 24.2 (23.2, 25.2) | 7.90 (7.81, 8.09) | 5.1 (4.4, 6.1) | 72.0 | 140.4 | 24.3 (23.6, 25.9) | 8.32 (8.21, 8.44) | 6.3 (5.2, 7.1) | 56.0 | 228.4 |
| | 50 % | 24.0 (23.5, 25.0) | 8.23 (8.16, 8.36) | 5.7 (5.1, 6.6) | 47.6 | 218.0 | 24.6 (22.9, 26.1) | 8.53 (8.37, 8.68) | 6.4 (5.4, 7.4) | 48.0 | 396.4 |
| | 100 % | 24.2 (23.6, 25.2) | 8.53 (8.38, 8.61) | 5.3 (3.7, 6.0) | 18.4 | 366.4 | 24.5 (23.1, 25.6) | 8.68 (8.60, 8.74) | 6.2 (5.1, 7.0) | 27.2 | 740.0 |
| Tank 4P (Treated) | 6.25 % | 24.4 (23.5, 25.7) | 7.84 (7.75, 7.99) | 5.3 (4.5, 6.2) | 68.0 | 91.2 | 24.6 (23.0, 25.6) | 7.94 (7.79, 8.04) | 6.2 (5.4, 7.0) | 64.8 | 104.4 |
| | 12.5 % | 24.1 (22.7, 24.9) | 7.73 (7.61, 8.01) | 5.6 (4.7, 6.5) | 66.4 | 106.8 | 24.7 (23.4, 25.8) | 8.00 (7.85, 8.15) | 6.1 (4.9, 6.8) | 62.0 | 146.0 |
| | 25 % | 24.3 (23.3, 24.9) | 7.89 (7.70, 8.15) | 4.9 (4.2, 6.2) | 57.6 | 148.4 | 24.4 (23.0, 25.7) | 8.29 (8.14, 8.42) | 6.3 (5.1, 6.9) | 57.6 | 233.6 |
| | 50 % | 24.4 (23.7, 25.3) | 8.19 (8.05, 8.41) | 5.0 (4.0, 6.4) | 42.8 | 226.0 | 24.6 (22.9, 26.8) | 8.53 (8.46, 8.68) | 6.0 (4.7, 6.9) | 46.4 | 405.2 |
| | 100 % | 24.8 (23.8, 25.7) | 8.51 (8.41, 8.64) | 4.8 (4.1, 5.8) | 18.0 | 382.0 | 24.6 (23.9, 25.8) | 8.68 (8.58, 8.74) | 6.2 (4.7, 7.2) | 26.4 | 775.6 |

[A] Dechlorinated Laboratory Water.

#### *3.6.2.3 Green Alga (Selenastrum capricornutum) Density*

Results from TC2 and TC3 WET tests involving the green alga *S. capricornutum* are presented in Table 24. There was no significant difference ($p<0.05$) in mean cell density between the algae exposed to effluent collected from treatment tanks 3P and 4P (all dilutions) and the algae exposed to the receiving water control, filtered water from Duluth-Superior Harbor (Table 24). There was also no significant difference ($p<0.05$) in mean cell density between the algae exposed to effluent (all dilutions) collected from treatment tanks 3P and 4P and the algae exposed to untreated effluent collected from tank 5P (Table 24).

Results for temperature and pH, measured daily, and dissolved oxygen, conductivity, hardness and alkalinity, measured only at the beginning of the test, are detailed in Table 25. Temperature was constant over the 96 hour test period, while pH ranged from a minimum of 7.23 in the TC2 performance control to a maximum of 9.63 in the 50 % effluent from TC2 tank 4P (Table 25). Hardness measured highest in the untreated effluent from tank 5P, while alkalinity, measured highest in the 100 % effluent from tanks 3P and 4P (Table 25).

Table 24. Cell density (Average ± Standard Error; n = 4) in a *Selenastrum capricornutum* whole effluent toxicity test after 96 hours exposure to treated and untreated ballast discharge collected during Test Cycles 2 and 3.

| Treatment Group | Exposure Solution | Test Cycle 2: Cells/mL | Test Cycle 3: Cells/mL |
|---|---|---|---|
| Performance Control[A] | N/A | $3.44 \times 10^6 \pm 1.87 \times 10^5$ | $3.6 \times 10^6 \pm 2.6 \times 10^5$ |
| Receiving Water Control | Filtered Duluth-Superior Harbor water | $2.62 \times 10^6 \pm 2.24 \times 10^5$ | $3.0 \times 10^6 \pm 1.0 \times 10^5$ |
| Tank 5P (Untreated) | 100 % | $2.48 \times 10^6 \pm 1.71 \times 10^5$ | $2.2 \times 10^6 \pm 1.7 \times 10^5$ |
| Tank 3P (Treated) | 6.25 % | $2.99 \times 10^6 \pm 1.82 \times 10^5$ | $3.0 \times 10^6 \pm 2.3 \times 10^5$ |
| | 12.5 % | $2.93 \times 10^6 \pm 1.79 \times 10^5$ | $3.3 \times 10^6 \pm 2.0 \times 10^5$ |
| | 25 % | $3.04 \times 10^6 \pm 1.90 \times 10^5$ | $3.5 \times 10^6 \pm 2.6 \times 10^5$ |
| | 50 % | $3.63 \times 10^6 \pm 1.79 \times 10^5$ | $3.6 \times 10^6 \pm 2.9 \times 10^5$ |
| | 100 % | $4.05 \times 10^6 \pm 3.31 \times 10^5$ | $3.4 \times 10^6 \pm 3.9 \times 10)$ |
| Tank 4P (Treated) | 6.25 % | $2.96 \times 10^6 \pm 3.60 \times 10^5$ | $3.2 \times 10^6 \pm 1.3 \times 10^5$ |
| | 12.5 % | $3.11 \times 10^6 \pm 2.64 \times 10^5$ | $3.6 \times 10^6 \pm 2.7 \times 10^5$ |
| | 25 % | $3.22 \times 10^6 \pm 1.38 \times 10^5$ | $3.6 \times 10^6 \pm 3.3 \times 10^5$ |
| | 50 % | $2.68 \times 10^6 \pm 5.02 \times 10^4$ | $3.3 \times 10^6 \pm 2.7 \times 10^5$ |
| | 100 % | $3.36 \times 10^6 \pm 2.33 \times 10^5$ | $3.2 \times 10^6 \pm 3.0 \times 10^5$ |

[A] USEPA Nutrient Culturing Media (U.S. Environmental Protection Agency Office of Water, 2002)

Table 25. Average (Minimum, Maximum) water chemistry parameters measured in exposure solutions during the *Selenastrum capricornutum* whole effluent toxicity tests for Test Cycles 2 and 3.

| Treatment Group | Exposure Solution | Test Cycle 2 | | | | | | Test Cycle 3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Temp. (°C) | pH | Dissolved Oxygen (mg/L) | Cond. ($\mu$S/cm) | Hardness (mg/L $CaCO_3$) | Alkalinity (mg/L $CaCO_3$) | Temp. (°C) | pH | Dissolved Oxygen (mg/L) | Cond. ($\mu$S/cm) | Hardness (mg/L $CaCO_3$) | Alkalinity (mg/L $CaCO_3$) |
| Performance Control[A] | N/A | 24.3 (22.6, 24.9) | 7.62 (7.24, 10.13) | 7.7 | 90.9 | 18.0 | 11.4 | 24.1 (22.6, 24.7) | 7.63 (7.23, 9.95) | 8.5 | 95 | 32.0 | 12.4 |
| Receiving Water Control | Filtered Duluth-Superior Harbor water | 24.4 (23.2, 25.1) | 8.31 (7.88, 9.57) | 8.1 | 272 | 86.0 | 73.8 | 24.2 (23.3, 24.7) | 8.16 (7.77, 9.52) | 8.2 | 255 | 82.8 | 68.0 |
| Tank 5P (Untreated) | 100 % | 24.5 (22.8, 25.2) | 8.51 (8.13, 9.45) | 8.2 | 464 | 148.0 | 119.8 | 24.2 (23.2, 24.7) | 8.45 (8.01, 9.07) | 8.2 | 470 | 178.8 | 162.8 |
| Tank 3P (Treated) | 6.25 % | 24.3 (23.1, 25.0) | 8.37 (8.08, 9.71) | 8.1 | 323 | 85.0 | 92.2 | 24.1 (23.2, 24.6) | 8.39 (7.96, 9.46) | 8.0 | 336 | 82.0 | 111.2 |
| | 12.5 % | 24.4 (23.1, 25.0) | 8.46 (8.10, 9.47) | 8.1 | 372 | - | - | 24.1 (23.2, 24.5) | 8.46 (7.98, 9.54) | 8.0 | 410 | - | - |
| | 25 % | 24.3 (23.0, 25.2) | 8.54 (8.14, 9.64) | 8.2 | 420 | - | - | 24.1 (23.1, 24.5) | 8.52 (8.00, 9.52) | 8.1 | 578 | - | - |
| | 50 % | 24.3 (23.0, 25.1) | 8.62 (8.15, 9.63) | 8.1 | 561 | - | - | 24.0 (23.1, 24.6) | 8.59 (8.02, 9.50) | 8.2 | 890 | - | - |
| | 100 % | 24.2 (23.0, 25.0) | 8.76 (8.26, 9.67) | 8.2 | 848 | 33.0 | 370.8 | 24.0 (23.4, 24.5) | 8.57 (7.96, 9.59) | 8.4 | 1483 | 41.2 | 760.0 |

Table 25. Average (Minimum, Maximum) water chemistry parameters measured in exposure solutions during the *Selenastrum capricornutum* whole effluent toxicity tests for Test Cycles 2 and 3 (Continued).

| Treatment Group | Exposure Solution | Test Cycle 2 | | | | | | Test Cycle 3 | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | Temp. (°C) | pH | Dissolved Oxygen (mg/L) | Cond. ($\mu$S/cm) | Hardness (mg/L $CaCO_3$) | Alkalinity (mg/L $CaCO_3$) | Temp. (°C) | pH | Dissolved Oxygen (mg/L) | Cond. ($\mu$S/cm) | Hardness (mg/L $CaCO_3$) | Alkalinity (mg/L $CaCO_3$) |
| Tank 4P (Treated) | 6.25 % | 24.6 (23.1, 25.0) | 8.45 (8.01, 9.59) | 8.1 | 312 | 84.0 | 92.8 | 24.0 (23.2, 24.5) | 8.40 (7.98, 9.51) | 8.1 | 338 | 80.0 | 112.8 |
| | 12.5 % | 24.6 (23.2, 25.0) | 8.50 (8.09, 9.52) | 8.1 | 351 | - | - | 24.2 (23.1, 24.9) | 8.49 (8.03, 9.52) | 8.1 | 415 | - | - |
| | 25 % | 24.6 (23.1, 25.2) | 8.59 (8.21, 9.55) | 8.1 | 427 | - | - | 24.1 (23.1, 24.7) | 8.58 (8.08, 9.44) | 8.1 | 582 | - | - |
| | 50 % | 24.5 (23.0, 25.0) | 8.73 (8.32, 9.63) | 8.0 | 574 | - | - | 24.1 (23.0, 25.0) | 8.62 (8.07, 9.62) | 8.2 | 897 | - | - |
| | 100 % | 24.5 (23.0, 25.0) | 8.85 (8.43, 9.50) | 8.0 | 880 | 31.0 | 388.0 | 24.1 (22.9, 24.9) | 8.67 (8.08, 9.55) | 8.5 | 1506 | 41.6 | 775.2 |

[A] USEPA Nutrient Culturing Media (U.S. Environmental Protection Agency Office of Water, 2002)

#### *3.6.2.4 Performance Controls and Stock Solutions*

TC2 and TC3 WET test performance controls met test acceptability criteria, indicating that the organisms were healthy prior to test initiation and not damaged during the test due to handling. The filtered Duluth-Superior Harbor water controls and untreated ballast water from tank 5P also met test acceptability criteria. The water chemistry of the *C. dubia* and *P. promelas* stock solution was measured daily prior to being used for renewal of each replicate exposure solution. Temperature, pH and dissolved oxygen did not vary greatly between the performance control, receiving water control, untreated effluent from tank 5P and the various dilutions of treated effluent from tanks 3P and 4P (Tables 26 and 27). Conductivity and alkalinity increased with greater concentrations of treatment effluent from tanks 3P and 4P, while hardness decreased with increasing concentrations of treatment effluent (Tables 26 and 27).

Table 26. Average (Minimum, Maximum) water chemistry results from measurements of stock solutions used during Test Cycle 2 whole effluent toxicity tests with *Ceriodaphnia dubia* and *Pimephales promelas*.

| Treatment Group | Exposure Solution | Temperature (°C) | Dissolved Oxygen (mg/L) | pH | Conductivity ($\mu$S/cm) | Hardness (mg/L $CaCO_3$) | Alkalinity (mg/L $CaCO_3$) |
|---|---|---|---|---|---|---|---|
| *C. dubia* Performance Control[A] | N/A | 24.4 (23.1, 26.6) | 7.4 (7.3, 7.7) | 8.26 (8.20, 8.31) | 567 (557, 575) | 168.0 | 114.8 |
| *P. promelas* Performance Control[B] | N/A | 25.7 (23.7, 27.9) | 6.0 (5.6, 6.4) | 7.37 (7.21, 7.51) | 140 (132, 152) | 52.0 | 49.0 |
| Receiving Water Control | Filtered Duluth-Superior Harbor water | 24.8 (23.9, 25.9) | 8.2 (7.8, 8.4) | 7.83 (7.65, 8.09) | 195 (183, 211) | 74.0 | 63.0 |
| Tank 5P (Untreated) | 100 % | 24.9 (24.1, 27.0) | 8.9 (8.2, 9.4) | 7.96 (7.82, 8.10) | 328 (323, 330) | 160.0 | 109.8 |
| Tank 3P (Treated) | 6.25 % | 24.9 (24.0, 25.9) | 8.1 (7.8, 8.5) | 7.91 (7.64, 8.09) | 232 (229, 235) | 69.0 | 82.6 |
| | 12.5 % | 24.9 (24.1, 25.8) | 8.0 (7.8, 8.4) | 7.96 (7.81, 8.09) | 266 (261, 268) | 67.0 | 102.4 |
| | 25 % | 24.9 (24.1, 25.8) | 8.2 (7.9, 8.5) | 8.05 (8.00, 8.12) | 340 (338, 341) | 58.0 | 137.8 |
| | 50 % | 25.0 (24.1, 26.3) | 8.2 (7.9, 8.5) | 8.13 (8.10, 8.19) | 486 (481, 489) | 45.0 | 210.8 |
| | 100 % | 25.3 (24.2, 27.1) | 9.2 (8.5, 10.0) | 8.19 (8.14, 8.24) | 776 (770, 783) | 20.0 | 359.0 |
| Tank 4P (Treated) | 6.25 % | 24.6 (23.9, 25.3) | 8.1 (7.8, 8.3) | 7.92 (7.76, 8.03) | 231 (222, 253) | 68.0 | 84.4 |
| | 12.5 % | 24.5 (24.1, 25.1) | 8.1 (7.9, 8.4) | 7.97 (7.88, 8.11) | 272 (269, 276) | 66.0 | 103.4 |
| | 25 % | 24.5 (24.2, 24.8) | 8.2 (7.9, 8.5) | 8.11 (8.05, 8.14) | 347 (342, 353) | 58.0 | 142.2 |
| | 50 % | 24.8 (24.4, 25.4) | 8.3 (8.1, 8.7) | 8.22 (8.15, 8.27) | 501 (495, 507) | 44.0 | 220.6 |
| | 100 % | 25.3 (24.5, 26.0) | 9.4 (8.6, 9.9) | 8.34 (8.29, 8.38) | 806 (790, 818) | 20.0 | 379.0 |

[A] Hard Reconstituted Culture Water
[B] Dechlorinated Laboratory Water
[C] Filtered Duluth-Superior Harbor Water

Table 27. Average (Minimum, Maximum) water chemistry results from measurements of stock solutions used during Test Cycle 3 whole effluent toxicity tests with *Ceriodaphnia dubia* and *Pimephales promelas*.

| Sample ID | Exposure Solution | Temp. (°C) | Dissolved Oxygen (mg/L) | pH | Conductivity ($\mu$S/cm) | Hardness (mg/L $CaCO_3$) | Alkalinity (mg/L $CaCO_3$) |
|---|---|---|---|---|---|---|---|
| *C. dubia* Performance Control[A] | N/A | 24.8 (24.1, 25.6) | 8.1 (7.8, 8.3) | 8.25 (8.20, 8.31) | 570 (564, 580) | 172.4 | 122.0 |
| *P. promelas* Performance Control[B] | N/A | 24.8 (24.7, 24.9) | 6.8 (6.4, 7.1) | 7.31 (7.23, 7.36) | 138 (135, 143) | 48.0 | 49.6 |
| Receiving Water Control | Filtered Duluth-Superior Harbor water | 25.2 (24.6, 25.9) | 8.5 (8.0, 9.0) | 7.75 (7.67, 7.81) | 174 (163, 182) | 70.4 | 62.4 |
| Tank 5P (untreated) | 100 % | 25.8 (24.3, 27.4) | 9.2 (8.1, 9.9) | 7.92 (7.88, 7.97) | 394 (388, 398) | 167.6 | 153.2 |
| Tank 3P | 6.25 % | 25.3 (24.2, 27.2) | 8.4 (7.9, 8.7) | 7.93 (7.85, 7.97) | 258 (251, 263) | 67.2 | 102.4 |
| | 12.5 % | 25.2 (24.3, 26.9) | 8.4 (8.0, 8.8) | 7.95 (7.87, 8.02) | 334 (326, 340) | 64.4 | 147.2 |
| | 25 % | 25.0 (24.3, 25.1) | 8.4 (8.0, 8.9) | 7.98 (7.89, 8.04) | 491 (486, 501) | 62.0 | 235.6 |
| | 50 % | 24.8 (24.1, 26.2) | 8.6 (8.1, 8.9) | 7.97 (7.93, 8.02) | 799 (789, 818) | 49.2 | 404.8 |
| | 100 % | 24.4 (23.6, 25.3) | 9.7 (8.5, 10.2) | 7.85 (7.81, 7.89) | 1403 (1387, 1417) | 30.4 | 747.6 |
| Tank 4P | 6.25 % | 25.2 (24.2, 27.2) | 8.4 (8.0, 8.8) | 7.95 (7.89, 7.98) | 257 (251, 263) | 66.0 | 107.6 |
| | 12.5 % | 25.1 (24.3, 26.8) | 8.4 (8.0, 8.7) | 8.01 (7.95, 8.06) | 337 (334, 341) | 64.4 | 148.8 |
| | 25 % | 24.9 (24.3, 26.7) | 8.5 (8.0, 8.8) | 8.06 (7.99, 8.12) | 500 (492, 510) | 58.0 | 238.0 |
| | 50 % | 24.6 (23.9, 25.7) | 8.7 (8.1, 9.1) | 8.03 (7.98, 8.06) | 820 (810, 825) | 48.0 | 416.8 |
| | 100 % | 24.1 (22.8, 24.8) | 9.8 (8.8, 10.6) | 7.99 (7.94, 8.05) | 1435 (1431, 1439) | 27.2 | 759.2 |

[A] Hard Reconstituted Culture Water
[B] Dechlorinated Laboratory Water

## 3.8 Quality Assurance/Quality Control

### 3.8.1 Calibration of Multiparameter Water Quality Sondes

Two YSI Sondes per TC were successfully calibrated according to the procedure outlined in *GSI/SOP/MS/G/C/1 - Procedure for Calibration, Deployment, and Storage of YSI Multiparameter Water Quality Sondes* (Table 28). For TC2, the conductivity in the intake samples and mock-treatment discharge samples was expected to be substantially lower than the conductivity in the treatment discharge samples. For this reason, two Sondes were used during TC2. The conductivity probe on one Sonde was calibrated using a low-conductivity standard (e.g., 996 $\mu$S/cm) and was to be used for intake and mock-treatment discharge sample measurements, while the probe on the second Sonde was calibrated with a high-conductivity standard (e.g., 9977 $\mu$S/cm) to be used for treatment discharge sample measurements. For TC3, the two Sondes were erroneously calibrated using the same conductivity standard, which was a low-conductivity standard (e.g., 994 $\mu$S/cm). Therefore, the calibration standard did not bracket the measured conductivity value in the treatment discharge tanks, and the conductivity data from tanks 3P and 4P on discharge is not reported.

Table 28. Dates of YSI 6600 V2-4 multiparameter water quality sonde calibration relevant to Test Cycles 1-4 of the *Project 41012*.

| Test Cycle | YSI Sonde | Date of Calibration | Calibrated By | Comments |
|---|---|---|---|---|
| 1 | GSI #3<br>GSI #4 | 20 July 2012 | Christine Polkinghorne | Calibration successful for both Sondes. |
| 2 | GSI #1<br>GSI #2 | 15 October 2012 | Christine Polkinghorne | Calibration successful for both Sondes. The conductivity probe on GSI #1 was calibrated using a low-conductivity standard (used for intake and mock-treatment Tank 5P discharge). The conductivity probe on GSI #2 was calibrated using a high-conductivity standard (used for treatment discharge Tank 3P and 4P). |
| 3 | GSI #1<br>GSI #2 | 9 August 2013 | Christine Polkinghorne | Calibration successful for both Sondes. GSI #1 and #2 were calibrated using the same conductivity standard, which did not bracket the measured conductivity values in the treated tanks on discharge. |
| 4 | GSI #2 (intake)<br>GSI #4 (intake)<br>GSI #1 (discharge) | 4 November 2013 (prior to intake); 11 November 2013 (prior to discharge) | Christine Polkinghorne and Kimberly Beesley | Calibration successful for both Sondes prior to intake and discharge. |

### 3.8.2 Data Quality Indicators

GSI used the following USEPA data quality indicators (where applicable) to determine compliance with data quality objectives: representativeness, accuracy, precision, bias, sensitivity, comparability and completeness. Data quality objectives and acceptance criteria for each of these indicators varied by analysis type and are described in *GSI/QAQC/QAPP/SB/1 - Quality Assurance Project Plan for Shipboard Tests* (GSI, 2013c).

#### *3.8.2.1 Water Chemistry*

Results of the data quality analysis for precision, bias, accuracy, comparability, completeness and sensitivity relative to water chemistry samples analyzed during TCs 1 through 4 intake and discharge are summarized in Table 29. All data quality objectives were met for TC1 (Table 29).

For TC2, the precision data quality objective was met for all parameters measured, except NPOC (Table 29). The bias data quality objective was not met for NPOC or DOC, as the filter blank and blank samples were on average greater than the LOQ (Table 29). As a result, the completeness objective for these two parameters was also not met (Table 29). All other TC2 quantitative and qualitative data quality objectives were met (Table 29).

For TC3, the precision data quality objective was met for all parameters, although an insufficient number of duplicates were analyzed for TSS, %T, and POM (Table 29). However, duplicate samples were not analyzed for NPOC and DOC and precision could not be determined (Table 29). The bias data quality objective was met for all blanks (Table 29). The completeness objective was not met for %T unfiltered, POM and MM, owing to not enough samples being collected from the drip sampler during tank 2P intake (Table 29). All other quantitative and qualitative data quality objectives were met for water chemistry analysis during TC3.

For TC4, the data quality objectives for precision, bias, and accuracy were met for all parameters (Table 29). The completeness objective was met for TSS, %T (filtered and unfiltered), POM, and MM (Table 29), however, this objective was not met for NPOC, DOC or POC because only two samples were collected during TVE#2 intake rather than three (Table 29). In addition, the sample container storing the first sample collected for NPOC/DOC analysis from TVE#3 intake broke during shipment. All other quantitative and qualitative data quality objectives were met for water chemistry analysis during TC4 (Table 29).

#### *3.8.2.2 Organisms ≥ 50 µm33*

The data quality assessment for organisms ≥ 50 µm during TCs 1 through 4 is presented in Table 30[34]. The quantitative data quality objective for bias was met for TC1 and TC3. During TC2, the bias data quality objective was met for percent taxonomic similarity but the relative percent difference of total number of live organisms was just outside the acceptance criteria at 21% RPD. For TC4, no QA counts were conducted on either of the discharge samples due to the truncated sampling and analysis plan, therefore, no data quality objective for bias could be determined. The precision data quality objective was met for TC1 – TC3. For TC4, the precision data quality objective was not met; the coefficient of variation was greater than 20 % for all of the samples likely because the density of live zooplankton in the intake and discharge samples was relatively low.

#### *3.8.2.3 Organisms ≥ 10 and < 50 µm*

The data quality assessment for organisms ≥ 10 and < 50 µm for TCs 1 through 4 is presented in Table 31. The quantitative data quality objective for bias and the qualitative data quality objective for comparability were achieved for TCs 1 and 2 (Table 31). For TC3, the quantitative data quality objective for bias (with regards to relative percent difference) was not achieved (Table 31), which is not surprising given the low density of organisms. The data quality objective for comparability was however achieved (Table 31). For

[33] Based on assumption that p3SFS flow meter was accurately recording flow rates.
[34] As above.

TC4, since no discharge samples were collected, the bias data quality objective could not be determined (Table 31). The data quality objective for comparability was achieved, however (Table 31).

Heat-killing was performed on TC 1-3 discharge samples to assess accuracy of the FDA stain approach at detecting live/dead with each specific assemblage. During the TC1 live assessment of heat-killed samples, small numbers of "live" algae from the green algae genera *Scenedesmus* and *Pediastrum*, ranging from 16 to 19 cells/mL, were present in the samples. These false positive live counts were likely artifacts of the heating process in combination with the FDA stain. If these taxa were being mischaracterized as alive due to heat-killing assessment, then the ETV DSP requires that the mischaracterized green algae density must be subtracted from the total density determined from the original non-heat-killed samples. However, almost no specimens of *Scenedesmus* and *Pediastrum* were observed as alive during the full assessment, so the incorrect live determination appeared to occur on specimens that died during the heat killing validation procedure. Although this discrepancy is not well understood, we do not believe it is justified to alter full discharge counts in response to it. TC2 and TC3 discharge samples revealed no "live" (i.e. stained, glowing green) organisms.

#### ***3.8.2.4*** *Organisms < 10 µm*

Data quality assessment results for organisms < 10 $\mu$m relative to TC3 are presented in Table 32. A data quality assessment was not conducted for this size class during TCs 1 and 2.

For TC3, the precision data quality objective was met for all analyses except *Enterococcus* spp. (Table 32). The diluent blank for heterotrophic bacteria analyzed from both the SimPlate and spread plate methods were positive, but at levels that did not affect the data. The accuracy data quality objective was not met for total coliform analysis (Table 32). The percent completeness data quality objective was met for all analysis types except total coliforms and total heterotrophic bacteria measured using the spread plate method (Table 32).

#### ***3.8.2.5*** *Whole Effluent Toxicity*

Data quality assessment results for TCs 2 and 3 WET tests are presented in Table 33. The data quality objective for *C. dubia,* the only species tested that has a requirement of monthly reference toxicant tests, was met for both TCs with the relevant reference toxicant tests resulting in an $LC_{50}$ value within the acceptance range (Table 33). For TC2, the performance control culture water for *C. dubia* met test acceptability for survival but not for reproduction (Table 33). All other quantitative and qualitative data quality objectives were met (Table 33).

Table 29. Data quality objectives, criteria, and results from water chemistry/quality analyses during Test Cycles 1-4. Values marked by an asterisk (*) did not meet GSI's data quality objective.

| Data Quality Indicator | Evaluation Process/ Performance Measurement | Data Quality Objective | Test Cycle 1: Performance Measurement Result | | Test Cycle 2: Performance Measurement Result | | Test Cycle 3: Performance Measurement Result | | Test Cycle 4: Performance Measurement Result | |
|---|---|---|---|---|---|---|---|---|---|---|
| Precision | Samples (10 %) are split in the laboratory analyzed in duplicate. Performance measured by average relative percent difference (RPD). | < 20 % average RPD. | Percentage of samples collected and analyzed in duplicate: 11 % | TSS: Results of duplicate analysis < MDL, and too low to use %T (filtered): 0.3 %. %T (unfiltered): 0.1%. NPOC: 4.4%. DOC: 5.2% | Percentage of samples collected and analyzed in duplicate: 13 % | TSS: 3.1 % %T (Filtered): 0.4 %. %T (Unfiltered): 0.2 %. NPOC: 84.2 %*. POM: 1.3 % | Percentage of samples collected and analyzed in duplicate = 8 %*; NPOC and DOC = 0 %* | TSS: 7.3%. %T (filtered): 0.2 %. %T (Unfiltered): 2.0 %. POM: 5.9% | Percentage of samples collected and analyzed in duplicate: 13 % | TSS: 3.2 %. %T filtered): 0.4 %. %T (unfiltered): 1.0 %. POM: 6.8 %. NPOC: 0.6 %. DOC: 2.0 % |
| Bias, Blanks and Filter Blanks | Deionized water samples (two per analysis date) filtered using the procedure outlined in *GSI/SOP/BS/RA/C/8*, and analyzed using the procedure outlined in *GSI/SOP/BS/RA/C/4.* | > 98 % average transmittance | Number of %T Filter blanks analyzed: 4 (2 per analysis date) | Filter blank (%T): 99.9 % | Number of %T filter blanks analyzed: 4 each (2 each per analysis date) | Filter blank (%T): 100.7 % | Number of %T Filter Blanks analyzed: 4 each (2 each per analysis date) | Filter blank (%T): 99.6 % | Number of %T Filter Blanks analyzed: 2 each | Filter blank (%T): 99.8 % |
| | Deionized water samples (two per analysis date) filtered, dried, and weighed following the procedure outlined in *GSI/SOP/BS/RA/C/8* | < 0.3 mg/L TSS (TC1); < 3.6 mg/L TSS (TC2); < 2.6 mg/L TSS (TC3 and TC4) | Number of TSS filter blanks analyzed: 4 (2 per analysis date) | Filter blank (TSS): 0.0 mg/L | Number of TSS filter blanks analyzed: 4 each (2 each per analysis date) | Filter blank (TSS): 0.0 mg/L | Number of TSS filter blanks analyzed: 4 each (2 each per analysis date) | Filter blank (TSS): 0.0 mg/L | Number of TSS filter blanks analyzed: 2 each | Filter blank (TSS): 0.0 mg/L |

Table 29. Data quality objectives, criteria, and results from water chemistry/quality analyses during Test Cycles 1-4. Values marked by an asterisk (*) did not meet GSI's data quality objective (Continued).

| Data Quality Indicator | Evaluation Process/ Performance Measurement | Data Quality Objective | Test Cycle 1: Performance Measurement Result | | Test Cycle 2: Performance Measurement Result | | Test Cycle 3: Performance Measurement Result | | Test Cycle 4: Performance Measurement Result | |
|---|---|---|---|---|---|---|---|---|---|---|
| Bias, Blanks and Filter Blanks (Cont.) | A blank prepared by acidifying a volume of deionized water to 0.2 % with concentrated hydrochloric acid and analyzed following the procedure outlined in *GSI/SOP/BS/RA/C/3*. | < 0.3 mg/L NPOC (TC1); < 0.4 mg/L NPOC (TC2); < 0.7 mg/L NPOC (TC3 and TC4). | Number of NPOC blanks analyzed: 8 (4 per analysis date) | Blank (NPOC): 0.2 mg/L | Number of NPOC blanks analyzed: 7 (3.5 per analysis date) | Blank (NPOC): 0.5 mg/L * | Number of NPOC Blanks analyzed: 6 (3 per analysis date) | Blank (NPOC): 0.48 mg/L | Number of POM filter blanks analyzed: 2 each | Blank (NPOC): 0.13 mg/L |
| | Deionized water samples (two per analysis date) filtered and analyzed following the procedure outlined in *GSI/SOP/BS/RA/C/3*. | < 0.5 mg/L DOC (TC1); < 0.4 mg/L DOC (TC2); < 0.7 mg/L DOC (TC3 and TC4) | Number of DOC filter blanks analyzed: 4 (2 per analysis date) | Filter blank (DOC): 0.4 mg/L | Number of DOC filter blanks analyzed: 4 each (2 each per analysis date) | Filter blank (DOC): 0.7 mg/L* | Number of DOC filter blanks analyzed: 4 each (2 each per analysis date) | Filter blank (DOC): 0.6 mg/L | Number of DOC filter blanks analyzed: 2 each | Filter blank (DOC): 0.3 mg/L |

Table 29. Data quality objectives, criteria, and results from water chemistry/quality analyses during Test Cycles 1-4. Values marked by an asterisk (*) did not meet GSI's data quality objective (Continued).

<table>
<tr><th>Data Quality Indicator</th><th>Evaluation Process/ Performance Measurement</th><th>Data Quality Objective</th><th colspan="2">Test Cycle 1: Performance Measurement Result</th><th colspan="2">Test Cycle 2: Performance Measurement Result</th><th colspan="2">Test Cycle 3: Performance Measurement Result</th><th colspan="2">Test Cycle 4: Performance Measurement Result</th></tr>
<tr><td rowspan="4">Accuracy</td><td>Samples (10 %) spiked with a total organic carbon spiking solution – with performance measured by average spike-recovery (SPR).</td><td>75 %-125 % average SPR.</td><td>Percentage of NPOC/DOC samples spiked: 16 %</td><td>NPOC/DOC: 99.8 %</td><td>Percentage of NPOC/DOC samples spiked: 27 %</td><td>NPOC/DOC: 96.9 %</td><td>Percentage of NPOC/DOC samples spiked: 13 %</td><td>NPOC/DOC: 100.4 %</td><td>Percentage of NPOC/DOC samples spiked: 12.5 %</td><td>NPOC: 101.2 % DOC: 100.3 %</td></tr>
<tr><td rowspan="3">Performance measured by average percent difference (%D) between all measured and nominal reference standard values.</td><td rowspan="3">< 20% average</td><td rowspan="3">Percentage of analysis days containing a reference standard: 100 %</td><td>TSS: 1.7 %</td><td rowspan="3">Percentage of analysis days containing a reference standard: 100 %</td><td>TSS: 2.5 %</td><td rowspan="3">Percentage of analysis days containing a reference standard: 100 %</td><td>TSS: 1.7 %</td><td rowspan="3">Percentage of analysis days containing a reference standard: 100 %</td><td>TSS: 0.7 %</td></tr>
<tr><td>NPOC reference standard: 0.7 %</td><td>NPOC, reference standard: 0.5 %</td><td>NPOC reference standard: 1.9 %</td><td>NPOC reference standard: 4.1 %</td></tr>
<tr><td>NPOC, 10 mg/L Standard: 1.7 %</td><td>NPOC, 10 mg/L standard: 1.8 %</td><td>NPOC 10 mg/L standard: 2.8 %</td><td>NPOC 10 mg/L standard: 2.2 %</td></tr>
<tr><td>Comparability</td><td>Routine procedures conducted according to appropriate SOPs to ensure consistency between test cycles.</td><td>Not applicable</td><td colspan="8">The following GSI SOPs were used for all water chemistry analyses conducted during the test cycles:<br>• GSI/SOP/BS/RA/C/3 - Procedures for Measuring Organic Carbon in Aqueous Samples.<br>• GSI/SOP/BS/RA/C/4 - Procedure for Determining Percent Transmittance (%T) of Light in Water at 254 nm.<br>• TC1 and TC2: GSI/SOP/BS/RA/C/8 - Procedure for Analyzing Total Suspended Solids (TSS).<br>•TC3 and TC4: GSI/SOP/BS/RA/C/8, v.3 – Procedure for Analyzing Total Suspended Solids (TSS), Particulate Organic Matter (POM), and Mineral Matter (MM)</td></tr>
</table>

Table 29. Data quality objectives, criteria, and results from water chemistry/quality analyses during Test Cycles 1-4. Values marked by an asterisk (*) did not meet GSI's data quality objective (Continued).

| Data Quality Indicator | Evaluation Process/ Performance Measurement | Data Quality Objective | Test Cycle 1: Performance Measurement Result | Test Cycle 2: Performance Measurement Result | Test Cycle 3: Performance Measurement Result | Test Cycle 4: Performance Measurement Result |
|---|---|---|---|---|---|---|
| Completeness | Percentage of valid (i.e., collected, handled, analyzed correctly and meeting data quality objectives) water chemistry samples measured out of the total number of water chemistry samples collected. Performance is measured by percent completeness (%C). | > 90 %C. | TSS: 18 valid samples/ 18 analyzed = 100 %C | TSS:23 valid samples/23 planned samples = 100 %C | TSS: 20 valid samples/22 planned samples = 91 %C | TSS: 12 valid samples/13 planned samples = 92 %C |
| | | | %T, Filtered: 18 valid samples/ 18 analyzed = 100 %C | %T, Filtered: 21 valid samples/21 planned samples = 100 %C | %T, Filtered: 18 valid samples/20 planned samples = 90 %C | %T, Filtered: 11 valid samples/12 planned samples = 92 %C |
| | | | %T, Unfiltered: 18 valid samples/ 18 analyzed = 100 %C | %T, Unfiltered: 17 valid samples/17 planned samples = 100 %C | %T, Unfiltered: 14 valid samples/16 planned samples = 88 %C* | %T, Unfiltered: 9 valid samples/10 planned samples = 90 %C |
| | | | NPOC: 18 valid samples/ 18 analyzed = 100 %C | NPOC: 27 valid samples/35 planned samples = 77 %C* | NPOC: 31 valid samples/32 planned samples = 97 %C | NPOC: 17 valid samples/19 planned samples = 89 %C* |
| | | | | DOC: 17 valid samples/21 planned samples = 81 %C* | DOC: 21 valid samples/22 planned samples = 95 %C | DOC: 10 valid samples/12 planned samples = 83 %C* |
| | | | | POC: 15 valid samples/15 planned samples = 100 %C | POC: 15 valid samples/16 planned samples = 94 %C | POC: 8 valid samples/10 planned samples = 80 %C* |
| | | | DOC: 18 valid samples/ 18 analyzed = 100 %C | POM:17 valid samples/17 planned samples = 100 %C | POM: 14 valid samples/16 planned samples = 88 %C* | POM: 11 valid samples/12 planned samples = 92 %C |
| | | | | MM: 15 valid samples/15 planned samples = 100 %C | MM: 14 valid samples/16 planned samples = 88 %C* | MM: 9 valid samples/10 planned samples = 90 %C |
| Sensitivity | The method detection limit (MDL) and limit of quantification (LOQ) for each analyte and analytical method utilized determined annually prior to the start of the testing season. | Not applicable | TSS MDL: 1.1 mg/L TSS LOQ: 3.6 mg/L | TSS MDL: 1.1 mg/L TSS LOQ: 3.6 mg/L | TSS MDL: 0.8 mg/L TSS LOQ: 2.6 mg/L | TSS MDL: 0.8 mg/L TSS LOQ: 2.6 mg/L |
| | | | | POM MDL: 0.5 mg/L POM LOQ: 1.5 mg/L | POM MDL: 0.6 mg/L POM LOQ: 2.0 mg/L | POM MDL: 0.6 mg/L POM LOQ: 2.0 mg/L |
| | | | NPOC MDL: 0.1 mg/L NPOC LOQ: 0.4mg/L | NPOC MDL: 0.1 mg/L NPOC LOQ: 0.4mg/L | NPOC MDL: 0.2 mg/L NPOC LOQ: 0.7 mg/L | NPOC MDL: 0.2 mg/L NPOC LOQ: 0.7 mg/L |
| | | | DOC MDL: 0.1 mg/L DOC LOQ: 0.4 mg/L | DOC MDL: 0.1 mg/L DOC LOQ: 0.4 mg/L | DOC MDL: 0.2 mg/L DOC LOQ: 0.7 mg/L | DOC MDL: 0.2 mg/L DOC LOQ: 0.7 mg/L |

Table 30. Data quality objectives, criteria, and results[35] from analyses of organisms ≥ 50 µm during Test Cycles 1-4. Values marked by an asterisk (*) did not meet GSI's data quality objective.

| Data Quality Indicator | Evaluation Process/ Performance Measurement | Data Quality Objective | Test Cycle 1: Performance Measurement Result | | Test Cycle 2: Performance Measurement Result | | Test Cycle 3: Performance Measurement Result | | Test Cycle 4: Performance Measurement Result | |
|---|---|---|---|---|---|---|---|---|---|---|
| Bias | 10% of treatment discharge samples and at least one intake per set of tests of a specific BWMS analyzed by two separate taxonomists – with performance measured by average percent similarity (PS) of taxonomic identification (live organisms only). | > 80 % average PS and < 20 % average RPD. | Percentage of treatment discharge samples analyzed by a second taxonomist: 1 out of 3 = 33 % | 91% PS and 4% RPD | Percentage of treatment discharge samples analyzed by a Second Taxonomist: 1 out of 3 = 33 % | 81 % PS and 21 % RPD* | Percentage of treatment discharge samples analyzed by a second taxonomist (2 out of 3): 67 % | 85 % PS and 10 % RPD | Percentage of treatment discharge samples analyzed by a second taxonomist : 0 %* | Cannot be determined; a second (quality assurance) count was not conducted on either of the discharge samples.* |
| Precision | Analyzed at least two subsamples from all samples analyzed via the "dead/total" counting method – with performance measured by coefficient of variation among subsamples (%CV) counted by the same analyst. | ≤ 20 % CV | Intake macrozooplankton: 17 %, n=3<br>Intake microzooplankton: 9 %, n=3<br>Discharge: 17 %, n=2 | | 15 %, n=5 | | 14 %, n=5 | | Intake: 27 %*; n = 3. Discharge macrozooplankton: 28 %*; n = 2. Discharge microzooplankton: 25 %*; n = 2. | |
| Comparability | Routine procedures are conducted according to appropriate SOPs to ensure consistency between tests. | Not applicable | The following GSI SOP was used for all zooplankton sample analyses conducted during the test cycles:<br>*GSI/SOP/MS/RA/SA/2 – Procedure for Zooplankton Sample Analysis* | | | | | | | |

[35] Based on assumption that p3SFS flow meter was accurately recording flow rates.

Table 31. Data quality objectives, criteria, and results from analyses of organisms ≥ 10 and < 50 µm during Test Cycles 1-4. Values marked by an asterisk (*) did not meet GSI's data quality objective.

| Data Quality Indicator | Evaluation Process/ Performance Measurement | Data Quality Objective | Test Cycle 1: Performance Measurement Result | | Test Cycle 2: Performance Measurement Result | | Test Cycle 3: Performance Measurement Result | | Test Cycle 4: Performance Measurement Result | |
|---|---|---|---|---|---|---|---|---|---|---|
| Bias | 10 % of treatment discharge samples and at least one intake sample per set of four test cycles analyzed by two separate taxonomists – with performance measured by average percent similarity (PS) of taxonomic identification (live organisms only) and average relative percent difference (RPD) of the total number of live organisms. | > 60 % average PS and < 20 % average RPD. | Percentage of Protist Samples Analyzed by a Second Taxonomist: 0 %* | Cannot be determined*; a second (QA) count was not conducted | Percentage of protist samples analyzed by a second taxonomist: 20 % (0/2 intake samples and 1/3 discharge samples) | PS: 96 % RPD: 0.3 % | Percentage of samples analyzed by a second taxonomist: 40 % (0 out of 2 intake samples and 2 out of 3 discharge samples) | PS: 85 % (average) RPD: 26 % (average)* | Percentage of samples analyzed by a second taxonomist: Not Applicable – There were no protist discharge samples collected. | Not Applicable – There were no protist discharge samples collected. |
| Comparability | Routine procedures are conducted according to appropriate SOPs to ensure consistency between tests. | Not applicable – Qualitative. | The following GSI SOP was used for all protist sample analyses conducted during the test cycles: *GSI/SOP/MS/RA/SA/1– Procedure for Protist Sample Analysis* | | | | | | | |

Table 32. Data quality objectives, criteria, and results from analyses of organisms < 10 µm during Test Cycles 1-3. Values marked by an asterisk (*) did not meet GSI's data quality objective.

| Data Quality Indicator | Evaluation Process/ Performance Measurement | Data Quality Objective | Test Cycle 1: Performance Measurement Result | | Test Cycle 2: Performance Measurement Result | | Test Cycle 3: Performance Measurement Result | |
|---|---|---|---|---|---|---|---|---|
| Precision | Samples (10 %) analyzed in duplicate – with performance measured by average relative percent difference (RPD) of all duplicate analyses. | < 30 % average RPD. | Not determined | Not determined | Not determined | Not determined | Percentage of samples analyzed in duplicate: 0*-13 % (dependent upon analysis type) | *E. coli*: 5 % RPD, n=3; Total Coliforms: 28 % RPD, n=3; *Enterococcus* spp.: 38 % RPD*, n=3; Heterotrophic SimPlate: 19 % RPD, n=4 |
| Bias, Operator | Samples (10 %) counted by two separate analysts – with performance measured by average RPD of all second counts. | < 20 % average RPD. | Not determined | Not determined | Not determined | Not determined | Percentage of samples counted by a second analyst: > 10 % (dependent upon analysis type) | *E. coli*: 1 % RPD, n=17; Total Coliforms: 2 % RPD, n=19; *Enterococcus* spp.: 0 % RPD, n=19; Heterotrophic SimPlate: 2 % RPD, n=26; Heterotrophic Spread Plate: 12 % RPD, n=59. |
| Bias, Positive Control | Qualitative positive control samples (American Type Culture Collection) analyzed on each analysis date. | Results must be greater than the limit of detection. | Not determined | | Not determined | | *E. coli*: All positive controls >1 most probable number (MPN)/100 mL, n=2; Total Coliforms: All positive controls >1 MPN/100 mL, n=2; *Enterococcus* spp.: All positive controls >1 MPN/100 mL, n=2; Heterotrophic SimPlate: All positive controls >1 MPN/mL, n=2; Heterotrophic Spread Plate: All positive controls >1 CFU/mL, n=2. | |

Table 32. Data quality objectives, criteria, and results from analyses of organisms < 10 µm during Test Cycles 1-3. Values marked by an asterisk (*) did not meet GSI's data quality objective (Continued).

| Data Quality Indicator | Evaluation Process/ Performance Measurement | Data Quality Objective | Test Cycle 1: Performance Measurement Result | Test Cycle 2: Performance Measurement Result | Test Cycle 3: Performance Measurement Result |
|---|---|---|---|---|---|
| Bias, Negative Control | Qualitative negative control samples (American Type Culture Collection) analyzed on each analysis date (note no negative control for Heterotrophic analyses). | Results must be less than the limit of detection. | Not determined | Not determined | *E. coli*:<br>All negative controls <1 MPN/100 mL, n=2;<br>Total Coliforms:<br>All negative controls <1 MPN/100 mL, n=2;<br>*Enterococcus* spp.:<br>All negative controls <1 MPN/100 mL, n=2 |
| Bias, Method/Procedural Blank | Filter-sterilized test water analyzed on each analysis date. | Results must be less than the limit of detection. | Not determined | Not determined | *E*. coli:<br>All method blanks <1 MPN/100 mL, n=2;<br>Total Coliforms:<br>All method blanks <1 MPN/100 mL, n=2;<br>*Enterococcus* spp.:<br>All method blanks <1 MPN/100 mL, n=2;<br>Heterotrophic SimPlate:<br>Intake blank <2 MPN/1 mL;<br>Discharge blank 12 MPN/1 mL*;<br>Heterotrophic Spread Plate:<br>Intake blank <1 CFU/1 mL;<br>Discharge blank 40 CFU/1 mL* |
| Bias, Diluent Blank | At least one day prior to sampling, diluents (sterile ballast or sterile deionized water) in growth media prepared and incubated overnight in order to determine sterility. | Results must be less than the limit of detection. | Not determined | Not determined | All diluent blanks negative for all *E. coli*/Total Coliform and *Enterococcus* spp. analyses.<br>Heterotrophic SimPlate:<br>Intake blank < 2 MPN/1 mL;<br>Discharge blank 12 MPN/1 mL*<br>Heterotrophic Spread Plate:<br>Intake blank < 1 CFU/1 mL;<br>Discharge blank 40 CFU/1 mL* |

Table 32. Data Quality Objectives, Criteria, and Results from Analyses of Organisms < 10 µm during Test Cycles 1-3. Values marked by an asterisk (*) did not meet GSI's data quality objective (Continued).

| Data Quality Indicator | Evaluation Process/ Performance Measurement | Data Quality Objective | Test Cycle 1: Performance Measurement Result | Test Cycle 2: Performance Measurement Result | Test Cycle 3: Performance Measurement Result |
|---|---|---|---|---|---|
| Accuracy | Quanti-cult®/Quanti-cult PLUS® samples (IDEXX Laboratories, Inc.) analyzed as a quantitative positive control at least once per ballast water treatment system test (note no quantitative positive control for Heterotrophic analyses). | *E. coli*: 65 – 263 MPN/100 mL; Total Coliforms: 33 – 103 MPN/mL; *Enterococcus* spp. 43 – 161 MPN/100 mL | Not determined | Not determined | *E. coli*: 98.8 MPN/100 mL; Total Coliforms: 27.5 MPN/100 mL*; *Enterococcus* spp.: 113.7 MPN/100 mL |
| Comparability | Routine procedures conducted according to appropriate SOPs to ensure consistency between tests. | Not applicable – Qualitative. | The following GSI SOPs were used for all microbial analyses conducted during the test cycles: *GSI/SOP/BS/RA/MA/1 – Procedure for Quantifying Heterotrophic Plate Counts (HPCs) using IDEXX's SimPlate® for HPC Method* *GSI/SOP/BS/RA/MA/3 – Procedure for the Detection and Enumeration of Enterococcus using Enterolert®* *GSI/SOP/BS/RA/MA/4 – Procedure for the Detection and Enumeration of Total Coliforms and E. coli using IDEXX's Colilert®* | | |
| Completeness | Percentage of valid (i.e., collected, handled, analyzed correctly and meeting data quality objectives) samples measured out of the total number of samples collected. Performance is measured by percent completeness (%C). | > 90 %C. | Not determined | Not determined | *E. coli*: 35 valid analyses/35 analyses total = 100 %C; Total Coliforms: 31 valid analyses/35 analyses total = 89 %C*; *Enterococcus* spp.: 34 valid analyses/35 analyses total = 97 %C; Heterotrophic SimPlate: 26 valid analyses/29 analyses total = 90 %C; Heterotrophic Spread Plate: 49 valid analyses/60 analyses total = 82 %C* |

Table 32. Data Quality Objectives, Criteria, and Results from Analyses of Organisms < 10 µm during Test Cycles 1-3. Values marked by an asterisk (*) did not meet GSI's data quality objective (Continued).

| Data Quality Indicator | Evaluation Process/ Performance Measurement | Data Quality Objective | Test Cycle 1: Performance Measurement Result | Test Cycle 2: Performance Measurement Result | Test Cycle 3: Performance Measurement Result |
|---|---|---|---|---|---|
| Sensitivity | The limit of detection (LOD) for the analytical method used is reported. | Dependent upon the analytical technique used. | *E. coli* LOD: < 1 MPN/100 mL; Total Coliforms LOD: < 1 MPN/100 mL; *Enterococcus* spp. LOD: < 1 MPN/100 mL; Heterotrophic SimPlate LOD: < 2 MPN/1 mL; Heterotrophic Spread Plate LOD: 0 CFU/1 mL | *E. coli* LOD: < 1 MPN/100 mL Total Coliforms LOD: < 1 MPN/100 mL *Enterococcus* spp. LOD: < 1 MPN/100 mL Heterotrophic SimPlate LOD: < 2 MPN/1 mL Heterotrophic Spread Plate LOD: 0 CFU/1 mL | *E. coli* LOD: <1 MPN/100 mL; Total Coliforms LOD: <1 MPN/100 mL; *Enterococcus* spp. LOD: <1 MPN/100 mL; Heterotrophic SimPlate LOD: <2 MPN/1 mL; Heterotrophic Spread Plate LOD: 0 CFU/1 mL |

Table 33. Data quality objectives, criteria, and results from whole effluent toxicity tests during Test Cycles 2 and 3. Values marked by an asterisk (*) did not meet GSI's data quality objective.

| Data Quality Indicator | Evaluation Process/ Performance Measurement | Data Quality Objective | Test Cycle 2: Performance Measurement Result | Test Cycle 3: Performance Measurement Result |
|---|---|---|---|---|
| Bias | Conducted monthly reference toxicity tests on *C. dubia* and determined the sensitivity of the test organisms relative to historical data using a quality control chart. | $LC_{50}$ value within two standard deviations of the historical mean $LC_{50}$. | *C. dubia* reference toxicant tests were performed monthly; the test relevant to TC2 was conducted 23 October 2012. LC50 = 421 mg/L KCl, which was within acceptance limits of 289 – 800 mg/L KCl. | *C. dubia* reference toxicant tests were performed monthly; the test relevant to TC3 was conducted 30 July 2013. $LC_{50}$ = 390 mg/L KCl, which was within acceptance limits of 289 – 800 mg/L KCl. |
| | A performance control, consisting of the optimal culture water for the species being tested, used to provide information on the health of the test organisms. Dechlorinated laboratory water was used for *P. promelas*, hard reconstituted water was used for *C. dubia* and algae growth media (USEPA, 2002) was used for *S. capricornutum*. | *C. dubia*: ≥ 80 % adult survival; 60 % of surviving adults must have ≥ three broods with an average total number of ≥ 15 young per female. *S. capricornutum*: Final cell density ≥ 1 x $10^6$ cells/mL and ≤ 20 %CV. *P. promelas*: ≥ 80 % survival; average dry weight per survivor ≥ 0.25 mg/fish | *C. dubia* adult survival: 80 % Number of broods: 10 % with three broods, 40 % with two broods, 60 % with one brood* Average total number young/female: 10* *S. capricornutum* final cell density: 3.4 x $10^6$ cells/mL. CV%: 11 % P. promelas survival: 97 % Average dry weight per survivor: 0.41 mg/fish | *C. dubia*: Adult survival: 100 % adult survival. 90 % with three broods. Average total number young/female: 24.6 *S. capricornutum*: Final cell density: 3.575 x $10^6$ cells/mL. CV%: 14.4 % *P. promelas*: Survival: 100 % Average dry weight per survivor: 0.413 mg/fish. |

Table 33. Data quality objectives, criteria, and results from whole effluent toxicity tests during Test Cycles 2 and 3. Values marked by an asterisk (*) did not meet GSI's data quality objective (Continued).

| Data Quality Indicator | Evaluation Process/ Performance Measurement | Data Quality Objective | Test Cycle 2: Performance Measurement Result | | Test Cycle 3: Performance Measurement Result | |
|---|---|---|---|---|---|---|
| Bias (Cont.) | Ensured a second, suitably-qualified operator analyzes at least 10 % of all experimental units. Performance measured by Relative Percent Difference (RPD). | < 10 % average RPD. | Percentage of *C. dubia* test chambers counted by a second person: 34 % of test chambers. Percentage of *S. capricornutum* test chambers counted by a second person: 23 % of test chambers. Percentage of *P. promelas* test chambers counted by a second person: 73 % of test chambers. | *C. dubia*: 0.3 % RPD *S. capricornutum*: 13 %* RPD *P. promelas*: 0.03 % RPD | Percentage of *C. dubia* test chambers counted by a second person: 54.5 % (average); Percentage of *S. capricornutum* test chambers counted by a second person: 10.9 % (average); Percentage of *P. promelas* test chambers counted by a second person: 87.5 % (average) | *C. dubia*: 1 % RPD *S. capricornutum*: 2 % RPD *P. promelas*: 0 % RPD |
| Precision | Duplicate samples from at least 10 % of the test chambers (during analysis of final cell density only) analyzed with performance measured by RPD of all duplicate analyses. | < 20 % average RPD. | Not calculated | | Duplicate analysis was conducted on 10.9 % of test chambers; RPD = 12 % | |
| Comparability | Routine procedures conducted according to appropriate SOPs to ensure consistency between tests. | Not Applicable – Qualitative. | The following GSI SOPs were used for all WET tests conducted during test cycles 2 and 3:<br>• *GSI/SOP/BS/RA/WET/1 - Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to Ceriodaphnia dubia*<br>• *GSI/SOP/BS/RA/WET/2 - Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to the Fathead Minnow (Pimephales promelas)*<br>• *GSI/SOP/BS/RA/WET/3 - Procedure for Assessing Chronic Residual Toxicity of a Ballast Treatment System to the Green Alga (Selenastrum capricornutum)* | | | |

### 3.8.3 Deviations from the Test/Quality Assurance Plans

Deviations from the TC 1 through 4 TQAPs are summarized in Table 34. The GSI PI deemed that none of these deviations were consequential to the quality of *Project 41012* BWMS evaluation findings. The source causes of the deviations did, however, help GSI generate suggestions for improvements to its implementation of the ETV DSP, and to the ETV DSP itself, including the p3SFS.

In TC1, unexpected ship operations forced several deviations from the intake and discharge sampling plan (Table 34). Specifically, the TC1 TQAP stated that four experimental ballast tanks would be sampled on intake and discharge to achieve sample water and analysis volume requirements, however, in light of substantial ballasting delays, the GSI team limited the experimental ballast tanks to three. This meant that on discharge, the rate of sample collection was increased to assure that the ETV DSP requirements were met. Other deviations from the TC1 TQAP were associated with operation of the p3SFS, retrieval of electronic data and measurement of POC instead of POM (Table 34).

In TC2, there were again deviations to the TQAP associated with IH ballast intake and discharge operations (Table 34). Specifically, tank 5P and 2P ballasting times were expected to be 70 to 100 minutes based on historical data, but were only 55 minutes and 52 minutes, respectively. Sufficient volumes of sample water were collected for analysis, however. Other deviations associated with TC2 included loss of the first 29 minutes of tank 2P's in-line, continuous data due to an operator error and two issues associated with replicate exposures in the WET tests (Table 34).

For TC3, deviations from the TC3 TQAP were associated with the number and/or type of samples collected (Table 34). Specifically, sample volumes collected from tank 2P on intake were lower than planned (Table 34). Sample collection was stopped 49 minutes into the ballast operation when the pressure differential of the p3SFS reached 5 psi. This interruption resulted in less water collected from the drip sampler such that only one replicate was available for water chemistry analysis, e.g., for analysis of TSS, %T and POM (Table 34). On discharge, the p3SFS's turbidity probe malfunctioned such that no data was available for any of three ballast tanks sampled (Table 34).

During TC4, deviations from the TQAP resulted from several causes (Table 34). First, the flow rate of the p3SFS drip sampler on both intake and discharge was significantly slower than expected. GSI personnel detected a crack in the plastic nipple of the p3SFS that leaked sample water. Though several attempts were made to repair the nipple, the drip sampler flow rate was still extremely slow. In addition, only two TVEs were collected during discharge operations instead of the planned three. TVE#2 sampling was stopped at 23:56 because IH deballasting ceased while the ship was waiting for additional cargo to load. The minimum wait time for deballasting to continue was three to six hours, which would have caused the sample collection team and analysts to time out. The GSI PI made the decision to abort sampling of TVE#3 as sampling the remaining discharge was not mission-critical. Finally, GSI staff had unexpected problems with sample containers and transport. Specifically, the temperature data logger was not placed into the cooler with the intake water chemistry samples and one of the sample collection containers storing a sample collected during intake for analysis of NPOC/DOC broke during shipment (Table 34).

Table 34. Summary of deviations to the Test Cycle 1 through 4 test/quality assurance plans.

| Test Cycle | GSI ID Number | Description of Deviation | Corrective Action | Potential Impact on Study |
|---|---|---|---|---|
| 1 | SB-ETV-01 | During tank 2P intake, the p3SFS was paused at 18:40 for approximately one minute to accommodate the ship's need to pause ballasting. After the pause, sampling was to resume. However, a technical issue with the p3SFS display resulted in the p3SFS not automatically starting. | Once the issue was discovered, the p3SFS sampling operation was re-started. At this point, the pump did automatically start and the sampling operation resumed. | The lack of flow to the p3SFS was not discovered for approximately 17 minutes, which resulted in the sample volume being 1.29 $m^3$ below that of the target volume. However, the ETV DSP states that a minimum sample volume of 20 L must be concentrated for untreated water (enumerated using dead/total count); the volume concentrated was well over 200x the required volume. |
| | SB-ETV-02 | There was no electronic data collected during ballast discharge due to an unformatted Micro SD card that the p3SFS would have used to store data. The manual states: "Upon boot-up of the controller, the operator will be notified if the Micro SD card is missing or improperly installed." Although the formatting instructions were provided, since the warning was not observed the operator made the incorrect assumption that the Micro SD card was ready for use. | The summarized operational data provided by the p3SFS after completion of each tank's ballast discharge operation was recorded by hand into a laboratory notebook and those data are used in this report. | There are no time stamped data for discharge flow rate, temperature, turbidity, pressure, pressure differential, pump frequency, and control valve position. Only what was provided in the summary files was recorded. |
| | SB-ETV-03 | A portion of Tank 5P intake and discharge operations were not sampled while GSI was attempting to get the p3SFS pump primed. It was observed that the M/V *Indiana Harbor* ballast system frequently functioned below the p3SFS's required pressure of 5 psi. From observation, it appears that the 5 psi requirement is only a requirement during start up and that once the p3SFS is primed it can function below that value. | GSI personnel requested the vessel's crew to temporarily increase the pressure in the main ballast line to allow for priming of the p3SFS pump. | The ship was able to accommodate the brief jump in pressure without affecting the cargo loading operation. |

Table 34. Summary of deviations to the Test Cycle 1 through 4 test/quality assurance plans (Continued).

| Test Cycle | GSI ID Number | Description of Deviation | Corrective Action | Potential Impact on Study |
|---|---|---|---|---|
| 1 (Cont.) | SB-ETV-04 | Three experimental ballast tanks were sampled on intake and discharge (i.e., port-side tanks 2P, 3P, and 5P) rather than four experimental tanks. 4P was not sampled. | The USCG STEP Sample Volume Calculator was used to determine the required discharge sample collection volume for the >50 $\mu$m size class, given that three samples could be combined in a single run rather than four. The discharge sample collection volume was then increased from 5.5 $m^3$ to 6.0 $m^3$ for each experimental ballast tank. | The total ballast volume sampled was less than planned; however, it was still many times greater than that required by the ETV DSP and the appropriate sample volume for three tanks was collected. |
| | SB-ETV-05 | Particulate organic carbon (POC = NPOC – DOC) was empirically measured, rather than directly measuring particulate organic matter (POM). | The POC concentration was reported, rather than the POM. | The ETV DSP states that the POM concentration is approximately two times the POC concentration. Therefore, TC1 POM concentrations can be estimated using the POC concentration. |
| 2 | SB-ETV-06 | Tank 5P and 2P intake p3SFS sample volumes were less than the target value of 6 $m^3$, and the drip sample volumes were less than the target value of 15 L. | No corrective action could be taken. Tank 5P and 2P ballasting times were expected to be 70 to 100 minutes based on historical data, but were only 55 minutes and 52 minutes, respectively. | The minimum zooplankton sample volume for untreated water is 20 L concentrated to 1 L, according to the ETV DSP. For TC2, 4.17 $m^3$ and 3.41 $m^3$ were sampled from tanks 5P and 2P, respectively. Therefore, the volumes collected greatly surpass the requirement. In addition, the drip sample volume collected was sufficient for all whole water samples. |
| | SB-ETV-07 | In-line, continuous data from the first 29 minutes of tank 2P intake operation was lost. The only in-line, continuous data available for tank 2P is from after the sampling pause. | The GSI Engineer estimated the volume of water sampled using the p3SFS based on the length of sampling pre-pause and the average flow rate. | The volume of water sampled using the p3SFS is an approximation based on the estimated volume sampled pre-pause and the measured volume sampled post-pause. |
| | SB-ETV-08 | Approximately 20 minutes into tank 4P's discharge operation, it was observed that the spigot on the drip sampler carboy was leaking. | Attempts to repair were unsuccessful. Instead GSI personnel switched to a functioning, clean 50 L carboy and drained the water from the leaking carboy into the new carboy. | A volume of water was lost from the integrated sample during the first 20 minutes of the sampling operation. This volume is unknown, but is relatively small in comparison to the 43 L collected in the integrated sample over the entire discharge operation. |
| | SB-ETV-09 | On WET test day 4, after siphoning exposure water from replicate beakers in the 12.5 % and 25 % - tank 3P treatment groups, renewal stock solution from 12.5 % was mistakenly poured into the 25 % - Tank 3P beakers. | GSI personnel made new 12.5 % - Tank 3P stock solution, and 90 % of the incorrect exposure water was siphoned from the 25 % - tank 3P solution and replaced with 25 % - Tank 3P stock solution. | The organisms in the 25 % - tank 3P treatment group were exposed to a lesser concentration of the tank 3P whole effluent for a very short time period (less than one hour). |

Table 34. Summary of deviations to the Test Cycle 1 through 4 test/quality assurance plans (Continued).

| Test Cycle | GSI ID Number | Description of Deviation | Corrective Action | Potential Impact on Study |
|---|---|---|---|---|
| 2 (Cont.) | SB-ETV-10 | On WET test termination day (Day 5), it was observed that there were two live organisms in tank 5P replicates 1 and 2 and zero live organisms (but no dead bodies) in 6.25 % - tank 3P replicates 1 and 2. It is likely that the organisms were inadvertently transferred to the incorrect replicate cups (Tank 5P, 1 and 2) on test day 4. | No corrective action could be taken, as the test was being terminated. The issue was documented on the datasheet. | Replicates 1 and 2 from the tank 5P group could not be factored into the number of young per female average. In addition, replicate 2 from the 6.25% - tank 3P group was not used to determine reproduction as it had not had three broods before the deviation occurred. |
| 3 | SB-ETV-11 | Sample volumes collected from tank 2P on intake were lower than planned because sample collection was stopped 49 minutes into the ballast operation due to the pressure differential of the p3SFS reaching 5 psi. This resulted in less water being collected from the “drip sampler” such that only one replicate was available for water chemistry analysis. | No corrective action could be taken. Tank 2P ballasting time was expected to be 70 to 100 minutes based on historical data. Sample collection was stopped at 49 minutes owing to the pressure differential of the p3SFS reaching 5 psi. However, at this point there was only 10 minutes of the ballast operation remaining, such that resuming sample collection was not feasible. | Water chemistry analyses were conducted on the one replicate available for testing. Results are comparable to those measured in the three replicate from tank 5P intake sampling. |
| | SB-ETV-12 | The p3SFS was not wired to the IH’s ballast main signal properly so ballast flow rates were not recorded continuously. | No corrective action could be taken. The issue was fixed as soon as possible, and prior to next sampling event. | Average flow rates were calculated by hand collected data taken about every 10 minutes. |
| | SB-ETV-13 | The drip sample flow rate for tank 4P discharge was above the target range of 23 to 33 L/Hr. | No corrective action could be taken. The expected ballast pumping duration for tank 4P was less than anticipated. To ensure enough volume for whole effluent toxicity (WET) testing, the drip sample flow rate for this tank was increased above the target range prior to the start of tank discharge. | 50 L of sample volume was collected by the drip sampler, well within the target range. |
| | SB-ETV-14 | The p3SFS’s turbidity probe malfunctioned on discharge such that no data was available for any of three ballast tanks sampled. | No corrective action could be taken. The probe was fixed as soon as possible, and prior to next sampling event. | Turbidity data was recorded by the YSI Multiparameter Water Quality Sonde. |
| | SB-ETV-15 | Not enough sample water collected for external collaborators. | No corrective action could be taken. The external collaborators were grateful for the samples which they received. | No impact. Samples for external collaborators were auxiliary to *Project 41012*. |

Table 34. Summary of deviations to the Test Cycle 1 through 4 test/quality assurance plans (Continued).

| Test Cycle | GSI ID Number | Description of Deviation | Corrective Action | Potential Impact on Study |
|---|---|---|---|---|
| 3 (Cont.) | SB-ETV-16 | A MadgeTech HiTemp 102 DataLogger (MadgeTech, Inc.; Warner, NH) was to be placed inside the cooler used to ship intake samples from Muskegon to Superior to automatically measure and record the temperature every 15 minutes during shipment and to ensure that the samples were maintained at ≤ 6 °C. The DataLogger was not placed with the samples inside the cooler. | The issue was communicated to the responsible staff. Retraining of the responsible staff was conducted. | Samples arrived in Superior as planned. Though ice cubes had melted in transit, samples were cool to touch. |
| | SB-ETV-17 | GSI Sonde #1 and #2 were calibrated prior to TC3 using the same low-conductivity calibration standard (i.e., 994 $\mu$S/cm), which did not bracket the conductivity in the treated tanks on discharge (i.e., tank 3P and 4P) | The conductivity data measured from tanks 3P and 4P are not reported. | The conductivity of the integrated water samples collected for WET testing was measured upon set up of the test, and these values can be used as an approximation of what the conductivity of the treated tanks were at the time of discharge. |
| 4 | SB-ETV-18 | The flow rate through the p3SFS drip sampler on intake and discharge was significantly slower than planned. GSI personnel detected a crack in the plastic nipple that was leaking water located just before the drip sampler shut off valve. | Assuming the leak was the problem leading to slow flow rates, GSI personnel attempted to repair the nipple, and reinstalled it prior to TC4 discharge, with negative results; the drip sampler flow rate was still extremely slow. After another attempt at repair, the GSI team communicated the issue and actions to the PI who directed the team to discontinue use the drip sampler for the remainder of TC4 as there was no obvious way to fix the problem, and any sample water would not be adequately quantitative. | No time-integrated protist samples were collected on discharge. In addition, the sample team collected grab samples for water chemistry at approximately the beginning, middle, and end of each intake sampling operation from a separate line off of the ballast main, except during TVE#2 intake when the sample collection team was unable to collect grab samples due to conflicting staffing demands. Two replicate samples were instead collected from the drip sampler carboy. |
| | SB-ETV-19 | The temperature data logger was not placed into the cooler with the intake water chemistry samples | The issue was communicated to the responsible staff. Retraining of the responsible staff was conducted. | Samples arrived as planned. Though the holding temperature of the samples during shipment is unknown, the cooler still had ice present upon arrival. |
| | SB-ETV-20 | The sample collection container storing the first grab sample collected for analysis of NPOC/DOC from TVE3 intake broke during shipment | Better packaging will be used for future shipments. | There are no NPOC/DOC measurements for TVE#3, however, NPOC/DOC data are available for TVEs #1 and #2 |

Table 34. Summary of deviations to the Test Cycle 1 through 4 test/quality assurance plans (Continued).

| Test Cycle | GSI ID Number | Description of Deviation | Corrective Action | Potential Impact on Study |
|---|---|---|---|---|
| 4 (Cont.) | SB-ETV-21 | Only two TVEs were collected during discharge instead of the planned three. TVE2 sampling was stopped at 23:56 because IH deballasting ceased while the ship was waiting for additional cargo to load. The minimum wait time for deballasting to continue was 3-6 hours, which would have caused the sample collection team and analysts to time out. The GSI PI made the decision to abort sampling of TVE#3 as sampling the remaining discharge was not mission-critical. | None. The issue is inherent to undertaking experiments on commercial operating vessels. | Two other TVEs were sampled. |
| Not Applicable | SB-ETV-22 | Following the end of the testing, it was determined that the p3SFS flow meter could not be successfully calibrated as installed. The team found that position changes of the upstream flow control valve affected the flow constant for the flow sensor. The flow meter was calibrated before its installation in the p3SFS, so the effect of the flow control valve was not detected. The location of the flow sensor installation did not agree with the manufacturer's specifications. The meter could not be successfully calibrated, and the TC1—TC4 data (flow and position ) was not adequate to permit the correction of the flow data. | All densities reported for the ≥ 50 $\mu$m size class are flagged in this report as estimates based on the assumption that the p3SFS was accurately measuring flow rate. | The accuracy of the sample volumes and flow rates measured by the p3SFS flow meter during *Project 41012* is unknown. |

# 4 LESSONS LEARNED AND SUMMARY

This section summarizes GSI's lessons learned from execution of the ETV DSP and the p3SFS over the four TCs, and provides recommendations for improvement based on these lessons. Some of the lessons learned are generally applicable to ETV shipboard tests; while others are specifically applicable to ETV shipboard tests of the partial NaOH BWMS installation onboard the IH. Through its implementation of *Project 41012*, the GSI team also identified ways to improve the ETV DSP, including the p3SFS, and expand its use for the verification of biological treatment efficacy and environmental acceptability of BWMSs.

## 4.1 ETV Draft Shipboard Protocol

Shipboard tests conducted according to the ETV DSP are expensive and time-consuming endeavors, and often TOs have a limited number of opportunities at achieving them. As a result, the ETV DSP should require a section within the TQAP for TO-anticipated problems and proposed TO measures to address them. The ETV DSP also should provide useful guidance for TOs in troubleshooting and resolving likely issues. Based on GSI experience with the ETV DSP in the Great Lakes, we identify the following examples of likely pitfalls and ways to avoid them.

### 4.1.1 Protecting Health and Safety of Personnel

The ETV DSP has a great deal of focus on data quality, and justifiably so. However, TO personnel health and safety is also a critical concern, intrinsically, and indirectly as it relates to data quality. Health and safety concerns arise in the ETV DSP around operation of the BWMS, but not around implementation of the shipboard tests. Based on GSI experience, there are critical areas in which TO personnel health and safety protection would benefit from explicit protocols embedded in the TQAP.

#### ***4.1.1.1*** *Personnel Overextension*

During implementation of the *Project 41012*, the overextension of GSI personnel was a major concern, especially when there were unexpected changes to IH ballasting operations resulting in delayed or prolonged sampling events. Even under routine circumstances, personnel were tasked with protracted sample collection and/or analysis of time-sensitive samples at all hours of the day/night and interstate travel to and from sampling events. Uneven port security systems, equipment failures, sudden changes in vessel operations and transport logistics for time-sensitive samples added to the stress of these events.

All four sampling events during *Project 41012* occurred overnight, with the earliest start in mid-afternoon and the latest completed late morning. After TC1, the GSI PI analyzed the actual (as opposed to planned) personnel effort associated with implementing the TC1 TQAP, and learned that some shift lengths for the GSI Test Manager, GSI Engineer and GSI Senior QAQC Officer were in excess of 18 hours—clearly unacceptable and unsustainable.

Accordingly the GSI PI set shift lengths between 8 and 12 hours during ship sampling events for TCs 2-4 (Figure 42) that included contingencies to provide additional staff in cases where the sampling schedules were delayed. For example, in TC4 only two TVEs were sampled during discharge instead of the planned for three; a six hour delay in IH cargo loading operations would have pushed GSI personnel over shift limits. Such decisions will continue to be part of the landscape for ETV DSP tests, and generally for shipboard tests.

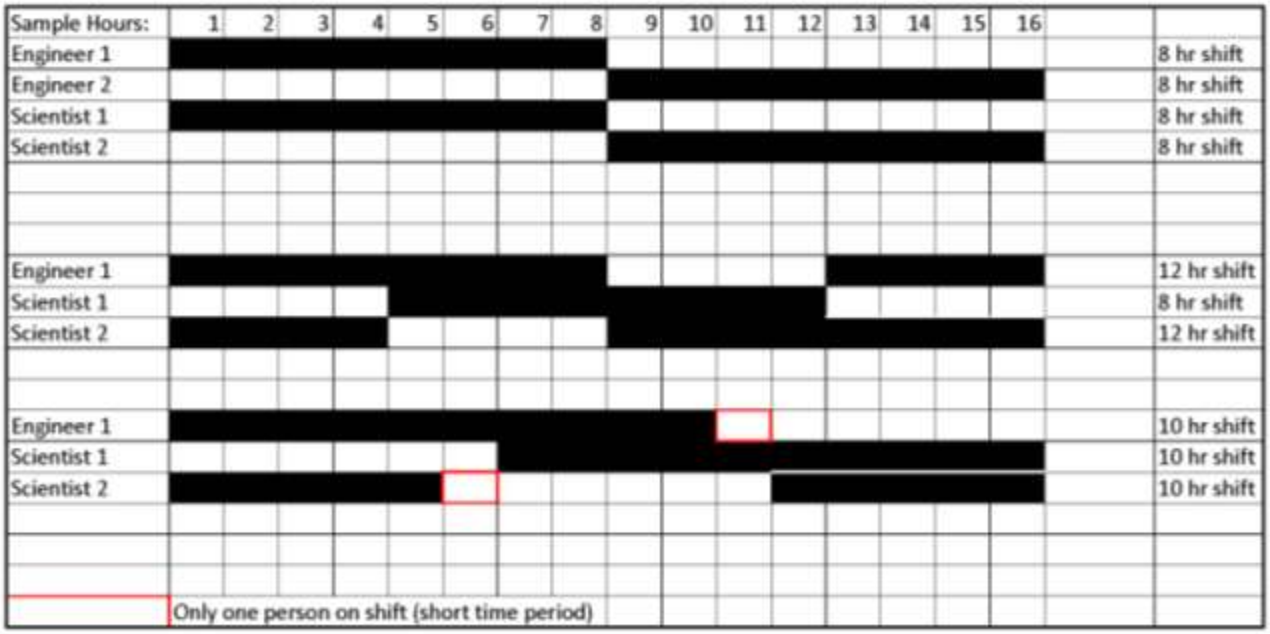



Figure 42. Proposed Test Cycle 2-4 GSI personnel hours.

**Recommendation:** Require that TOs explicitly demonstrate in the TQAP how they will protect personnel health and safety in terms of preventing overextension, while maintaining data quality. Specifically, ideal effort guidelines for personnel should be developed and stated in the TQAPs, and an adequate pool of qualified personnel should be included to account for unprogrammed staffing needs that may occur, such as those associated with ship schedule delays. These guidelines should be similar to Data Quality Objectives in the QAQC system, and the QAQC audit should compare outcomes against them in the same way.

#### *4.1.1.2 Exposure to Harmful Substances and Organisms*

During TC1, GSI personnel noted that the first tank to receive ballast water had extremely high loads of sediment produced by the action of the ship's propellers on the bottom as it maneuvered into its berth. The sediments clogged the sampling nets and made the microscopic analysis of samples more difficult. For subsequent tests, the IH crew filled experimental tanks later in the ballasting process. The IH crew routinely employs best management practices to reduce the amount of sediment uploaded into the vessel during ballast intake operations, for example, by raising the position of the vessel's sea chests. Ballasting order decisions are not typically influenced by pollution or sampling staff health and safety concerns, however. Concern over exposure to sediment and clogging of sampling equipment could negatively impact TC validity, by reducing physical/chemical challenge water conditions to below ETV DSP requirements.

The presence of sediment in samples may also pose a health threat to the sampling team when the ballast intake occurs in harbors with polluted waters and sediments. The TC1 intake sampling took place in Hammond, Indiana, in the Grand Calumet River Area of Concern (AOC). This AOC notably has contaminated sediments, including PCBs, PAHs and heavy metals such as mercury, cadmium, chromium and lead (USEPA, 2014). Ports overseas may also have high pathogen concentrations that would pose a health threat to sampling personnel.

**Recommendation:** Require that TOs document in the TQAPs how personnel will be protected from exposure to harmful substances and organisms in ballast water**.** Protection plans should include proper attire and safeguard for handling hazardous liquids and ballast operational planning to reduce sources of exposure.

### 4.1.2 Managing Sampling Logistics

#### *4.1.2.1 Unplanned Changes to Ballast Flow Rates*

Ballasting rates were highly variable during the four tests, in response to vessel loading and unloading requirements, cargo operations and crew decisions. This variability made it impossible to accurately predict with certainty sampling duration and p3SFS and drip sampler flow rates. During *Project 41012*, the TO sampling parameters did not adequately hedge against such variability, leading to specific operational parameters not being within their valid range.

For example, a vessel crew operational decision in TC2 to deballast less water than expected resulted in an abbreviated deballasting time and the target operational condition for total volume sampled from tank 3P not being met. To compensate for the reduced deballasting time, GSI personnel increased the flow rate of the drip sampler, thereby ensuring that an acceptable volume of whole water was collected. However, this decision resulted in the drip sampler flow rate being above the target range for this specific TC.

**Recommendation:** Provide guidance to TOs to include safety margins in their TQAP experimental designs. TQAPs should contain explicit safety cushions in valid range calculations to assure that sufficient volumes for statistical certainty will be collected even if ballast flow rates and durations are altered. The ETV DSP should provide for and recommend a sample volume cushion in the sample collection design.

#### *4.1.2.2 Sample/Ballast Flow Proportionality*

Section 5.4.3.1.1 of the ETV DSP states "…it is critical that 'flow proportional' samples for analysis be obtained during the entire filling and emptying of the ballast tanks under study." Ballast rates often vary as ballast tanks fill/drain, as the ship rises and ballast pump height changes in relation to the water line, and especially when the ship powers down and restarts its pumps during a ballasting operation. Indirect assessment of ballast rates using rates of change in ballast tank heights have a significant lag time. The sample flow rate to ballast system flow rate is difficult to determine under these circumstances in real time. The flow rate or its indirect measures must be calculated after the fact, making proportionality ultimately a matter of chance. In the end, the 'flow proportional' condition cannot be reliably accomplished without a reliable *in situ* ballast flow monitoring capability, which many ballast systems will have but some may not, and a reliable flow meter and flow control apparatus on the sampling system. In addition, TO sampling systems' mechanisms for meeting target sample flow rates and volumes, and any sensors they employ to maintain sample flow proportionality with the ballast main flow, must be well-calibrated and validated prior to use in ETV DSP testing. GSI's assessment of BWMS effectiveness at inactivating zooplankton in these tests was severely compromised by a malfunctioning flow control/meter apparatus in the p3SFS, a matter only discovered after the tests.

**Recommendations:** Define acceptable limits for how far the sampling system can stray from proportional sampling so that at a minimum the TO can determine *post facto* whether to disqualify the test on the grounds of disproportionality. GSI also recommends that the ETV DSP provide guidance as to how to best measure against the limits and at what frequency. If tank heights are to be recorded, the data should be collected and recorded every 5 minutes or less, and time recorded to seconds in order to give a useful estimate of the ballasting rate. Sampling system flow meters and control equipment should be empirically validated, and flow meters should be calibrated, at a land-based testing facility prior to installation on the ship. Installation-related problems should be assessed prior to commencement of ship testing.

#### *4.1.2.3 Clarifying "Whole Tank" vs. "Partial Tank" Sampling Requirement*

The experimental design contained in the ETV DSP requires that the same water in each ballast tank that was sampled on intake should also be sampled on discharge to assure that challenge conditions are known and met for experimental water (USEPA, 2012). As noted above, Section 5.4.3.1.1 of the ETV DSP states "…it is critical that … samples for analysis be obtained during the entire filling and emptying of the ballast tanks under study." This requirement is supported by First *et al*. (2013) which describes the stratification of organisms in land-based tanks and suggests collecting multiple time-integrated samples throughout the discharge event. However, it is not clear that sampling a whole tank top to bottom is requisite to representativeness in the actual shipboard environment. Moreover, in many cases, ship operational constraints may preclude it. For *Project 41012*, for example, sampling whole tanks meant that GSI could perform tests on fewer voyages because coal was the only cargo load that allowed the ship to deballast tanks completely. Sampling the entire contents of experimental ballast tanks on intake, and a portion of the experimental ballast water on discharge, regardless of which tanks and what proportion of those tanks, is another possibility that would allow more flexibility for ETV-consistent ship trials.

**Recommendation:** Validate the assumption that partial tank sampling gives different results from whole tank sampling on board a ship.

#### *4.1.2.4 Requiring a Qualitative Determination for Whole Effluent Toxicity (WET) on Intake*

The ETV DSP experimental design does not require WET testing of ballast intake. Problems could arise, however, if a BWMS developer fails an ETV DSP validation process as a result of apparent residual effluent toxicity when the cause was with harbor water quality at the point of uptake.

**Recommendation:** Require that TOs provide some sort of evidence from the literature, or if necessary from new empirical tests, to eliminate intake water toxicity as a source for WET post BWMS treatment.

#### *4.1.2.5 Updating Isokinetic vs. Sub-Isokinetic Sampling Requirement*

Guidance documents are ambiguous regarding the isokinetic sampling. For example, in Section 5.4.3.1.2 of the ETV DSP an isokinetic sample port is suggested and this recommendation is expanded upon in Appendix B of the protocol (USEPA, 2012). However, Appendix B does not recommend isokinetic sampling but a specific sub-isokinetic range.

**Recommendation:** Update the ETV DSP text to consistently recommend the same sub-isokinetic range.

### 4.1.3 Requirements around Challenge Conditions

#### *4.1.3.1 Sedimentation*

The ETV DSP states that the TQAPs must include locations for ballasting that will have high likelihoods of producing sufficiently challenging natural waters for testing subject BWMSs. The TQAPs must also provide the rationale to support the location selections. TO selection of specific intake locations is often not an option, however. Also, vessels regularly undertake best management practices to reduce the amount of sediment uploaded during ballast intake operations, including by raising the position of the vessel's sea chests. These decisions, though beneficial in many ways, may result in challenge water parameters not meeting ETV DSP targets.

Maintaining a strict challenge condition for ship test cycles with respect to TSS and POC/POM could lead to the invalidation of many useful shipboard tests. For example, TC1 would have been invalid on these grounds as the IH crew employed best management practices to reduce the amount of contaminated sediment in the ballast water intake.

**Recommendation:** Since high levels of TSS and POC/POM are required in ETV land-based testing where water chemistry manipulation is feasible, ETV DSP requirements for meeting these water chemistry parameters should be removed and the values simply measured and reported.

#### *4.1.3.2 Living Organisms*

With regard to living organisms, for the ≥ 50 $\mu$m size class the ETV DSP permits TOs to assume 80 % of organisms in intake are alive, so that live/dead analysis can be by-passed. The idea is sound as it will save many hours of unnecessary analysis. On the other hand, the assumed percent live may be overly liberal. For example, in TC1, the live zooplankton fractions in tanks 2P, 3P and 5P were 78, 76, and 72 % , respectively on intake. In TC2, 84 % of the zooplankton were alive in tank 5P and 73 % in tank 2P, for an average of 79% live density. In TC3 only 41 % to 59% of the zooplankton in intake samples were alive. During TC4 intake, the percentage of live zooplankton in the samples ranged from 43 % to 63 %.

In the ≥10 $\mu$m and <50 $\mu$m size class, only two of the four *Project 41012* TCs met the ETV DSP target minimum for 500 cells/mL live organisms, suggesting that this minimum may be hard to meet in all cases.

**Recommendation:** Lower the presumed percent live for the ≥ 50 $\mu$m size class in preserved intake samples, and allow a higher presumption only with seasonal validation. GSI also recommends that the ETV DSP soften the requirement that at least four TCs meet biological challenge condition targets to three in the case of the ≥10 $\mu$m and <50 $\mu$m size class.

#### *4.1.3.3 Particulate Organic Matter and Particulate Organic Carbon Relationship*

The ETV DSP states that "POM concentration is generally about twice the POC concentration". Based on this assumption, TOs could measure POC in place of POM, which requires more analysis effort. Based on the data collected during TCs 1-3 intake and discharge and TC4 intake, the ETV DSP assumption does not appear to be correct for the harbors that were sampled. There did not appear to be a consistent relationship between POM and POC, and in all cases POC concentrations were less than half the POM concentration.

**Recommendation:** Revisit the assumption that POM concentration is twice that of POC and require that POM be measured, rather than POC as a surrogate, to assess the challenge water conditions.

## 4.2 The p3SFS

### 4.2.1 Hardware

#### *4.2.1.1 Flow Sensor*

The flow meter on the p3SFS reported inaccurate flow rates. The movement of the p3SFS's flow control valve caused inaccurate reading of the sample flow meter. The sample volume as well as proportionality with ballast flow is determined using the p3SFS flow meter.

**Recommendation:** Install the flow meter further downstream from major flow disturbances such as pumps, control valves and bends. Most flow meter manufacturers provide guidance on these distances. Also, calibrate the flow meter while it is installed in the p3SFS at a minimum two different flow rates.

#### *4.2.1.2 Additional Sample Ports*

In the case of the IH two sample ports were required to alleviate contamination issues given the partial installation of the BWMS, however, vessels with more complex ballast systems also may require more than just a single

intake and single discharge sampling location. As illustrated during TC 1-4 on the IH, moving the sample ports after the vessel has taken on ballast can be a difficult and sometimes dangerous process for vessel crews.

**Recommendation:** Supply enough sample ports so that test vessel crews are not required to move the ports after the vessel has taken on ballast. The hoses supplying each port should be switchable without unbolting the port flange from the ballast main.

#### *4.2.1.3 Differential Pressure Sensor*

The p3SFS's differential pressure sensor is unreliable. The manifold for the differential sensor had to be reset at the start and end of each TC. The supply lines to the manifold also create a contamination concern as they could potentially hold water from previous uses. Pressure sensors are already installed at the inlets of each of the p3SFS's filter canisters.

**Recommendation:** Install a second pressure sensor downstream of the canister to calculate the differential pressure across the canister. This would give the pressure in the canister plus the differential over the canister without the contamination risk of the supply lines to the manifold. It would also remove operational steps dealing with the manifold.

#### *4.2.1.4 p3SFS Pump Type*

The operating range of the p3SFS is limited because the system must be primed by the ballast line.

**Recommendation**: Switch the p3SFS pump to a self-priming unit to expand the range of conditions in which the sampling system can operate.

#### *4.2.1.5 Filter Sock Construction*

An excessive amount of silicone was used to seal the seams of the p3SFS filter socks, creating an area of refuge for live organisms that is difficult to rinse. In addition, the filter sock rinsing procedure requires that the operator place their hand and arm inside the sock in order to turn it inside out for the final rinsing. Accidental contact with the sample during this procedure may affect the sample's integrity, and poses the hazard of contact between the operator and contaminated sediments in the sample.

**Recommendation:** Seal the p3SFS filter socks with a thinner bead of silicone, and include a cup at the bottom of the sock (similar to a meshed cod end) that would collect the concentrated sample. The sock could be rinsed into this cod end, which can then be removed and rinsed into the sample container.

#### *4.2.1.6 Drip Sample Collection*

The WET testing performed during TC 2 and 3 discharge sampling following BWMS treatment required a sample volume up to 50 L. The 50 L carboy was too large for GSI personnel to safely invert to mix the sample.

**Recommendation:** Modify the p3SFS to allow collection of two drip samples simultaneously into two 19 L carboys. Care would have to be taken to assure quantitative equivalency of the paired.

#### *4.2.1.7 Grab Sample Collection*

The collection of discrete grab samples is not possible using the current version of the p3SFS. Since discrete grab samples are required by the ETV DSP it makes sense to have the sampling system be able to take grab samples without the need of a separately installed sample port on the ballast main.

**Recommendation:** Modify the p3SFS to provide for collection of discrete grab samples.

### 4.2.2 Software

#### *4.2.2.1 Turbidity Sensor*

The p3SFS in-line turbidity sensor reads (i.e., on the control screen) and reports (in the auto-logged data) that it is measuring TSS and with data reported in "mg/L", when in fact, the unit is reporting readings in NTU.

**Recommendation:** Modify the p3SFS control screen and auto-log so that this sensor is measuring turbidity in NTU.

#### *4.2.2.2 Secure Digital (SD) Card Error Reporting*

No electronic data were collected during the TC1 discharge event because the Micro SD card used by the p3SFS to store data was not formatted. The manual states: "Upon boot-up of the controller, the operator will be notified if the Micro SD card is missing or improperly installed." No notification was given, so the operator believed that the Micro SD card was ready for use.

**Recommendation:** Modify the p3SFS's SD card error screen to require user interaction to clear. In this situation, if a SD card is not formatted properly the user would be alerted.

#### *4.2.2.3 Turbidity Sensor*

GSI personnel observed that the turbidity sensor of the p3SFS was prone to quick swings during deballasting and that during one TC's discharge operation, the sealant ring on the cable leading into the unit was unexpectedly far from the body of the unit indicating that it could have been pulled from the unit.

**Recommendation:** Investigate and/or repair the p3SFS turbidity sensor.

#### *4.2.2.4 Accuracy of Temperature and Turbidity Sensor Measurement Data*

GSI personnel observed differences in temperature and turbidity values results depending upon the data output type and measurement method. These differences raised concern about the accuracy of the temperature and turbidity data provided by the p3SFS, as four distinct sets of data were derived from the same parameters. The following data types were collected and compared after TC1:

1. *In situ* continuous data automatically logged every second by the p3SFS and saved to the SD card. The output of this data type was an Excel spreadsheet, and basic descriptive statistics (i.e., average and standard deviation) were performed by GSI.
2. *In situ*, continuous temperature and turbidity data provided as a summary by the p3SFS (i.e., basic descriptive statistics performed by the p3SFS) and hand-recorded by GSI at the end of each sampling operation.
3. Hand-recorded data from the p3SFS AquaSensors display, which was connected to the temperature and turbidity sensors and provided real-time data. Basic descriptive statistics were performed by GSI on these data.
4. Measurement data from GSI's YSI Multiparameter Water Quality Sonde. The temperature and turbidity (among other water quality parameters) were measured by GSI on the integrated (drip) sample. This can be considered a time-integrated average of the entire sampling operation.

After completion of TC1, means from the four data sources were compared. As shown in Table 35, similar results were achieved from the continuous, *in situ* electronic data and the p3SFS-averaged data summary. However, in all cases, the hand-recorded AquaSensors measurements averaged higher temperature values than the p3SFS data (Table 35). The temperature values measured by GSI in the integrated sample were similar to the AquaSensors measurements, but were still higher than the p3SFS measurements (Table 35).

Table 35. Test Cycle 1 - comparison of temperature results measured using the p3SFS data log (average of in-line, continuous data), p3SFS data summary (average provided at end of each operation), p3SFS AquaSensors display (average of hand-recorded measurements), and GSI YSI Multiparameter Water Quality Sonde (from integrated sample).

| Data Type | Average Temperature (°C) | | | | | |
|---|---|---|---|---|---|---|
| | 5P Intake | 2P Intake | 3P Intake | 2P Discharge | 3P Discharge | 5P Discharge |
| p3SFS Data Log (In-Line Continuous, Electronic Data) | 24, *n*=4898 | 24, *n*=3746 | 24, *n*=5033 | No logged data. | No logged data. | No logged data. |
| p3SFS Summary (In-Line Continuous, Hand Recorded) | 24.22, *n* not reported | Summary did not produce reliable data. | 24.28, *n* not reported | 21.61, *n* not reported | 21.44, *n* not reported | 21.22 *n* not reported |
| p3SFS AquaSensors Display (In-Line Continuous, Hand Recorded) | 28.6, *n*=3 | 29.1, *n*=2 | 28.5, *n*=3 | 24.5, *n*=7 | 24.5, *n*=3 | 24.6, *n*=4 |
| GSI Measurement (Integrated) | 31.76, *n*=1 | 31.78, *n*=1 | 30.52, *n*=1 | 25.22, *n*=1 | 25.18, *n*=1 | 25.22, *n*=1 |

As shown in Table 36, in all cases the AquaSensors turbidity measurement data averaged lower than the p3SFS turbidity measurements. The turbidity measured by GSI from the integrated sample was different from both the p3SFS data and the AquaSensors data (Table 36).

Table 36. Test Cycle 1 - comparison of turbidity results measured using the p3SFS data log (average of in-line, continuous data), p3SFS data summary (average provided at end of each operation), p3SFS AquaSensors display (average of hand-recorded measurements), and GSI YSI Multiparameter Water Quality Sonde (from integrated sample).

| Data Type | Average Turbidity (NTU) | | | | | |
|---|---|---|---|---|---|---|
| | 5P Intake | 2P Intake | 3P Intake | 2P Discharge | 3P Discharge | 5P Discharge |
| p3SFS Data Log (In-Line Continuous, Electronic Data) | 25, *n*=4898 | 12, *n*=3746 | 7, *n*=5033 | No logged data. | No logged data. | No logged data. |
| p3SFS Summary (In-Line Continuous, Hand Recorded) | 26, *n* not reported | Summary did not produce reliable data. | 8, *n* not reported | 7, *n* not reported | 6, *n* not reported | 16 *n* not reported |
| p3SFS AquaSensors Display (In-Line Continuous, Hand Recorded) | 25.730, *n*=3 | 7.981, *n*=2 | 5.751, *n*=3 | 3.682, *n*=7 | 1.449, *n*=3 | 0.738, *n*=4 |
| GSI Measurement (Integrated) | 8.5, *n*=1 | 6.0, *n*=1 | 5.1, *n*=1 | 2.8, *n*=1 | 2.5, *n*=1 | 2.2, *n*=1 |

**Recommendation:** Conduct validation experiments to determine the most accurate inline sensors for temperature and turbidity, as well as the data output type for the p3SFS that produces the most accurate and reliable results. Moreover, calibration activities should be performed on *in situ* measurement devices prior to ETV DSP tests, and if possible, between TCs. Alternatively, if in-line sensor installation cannot be routinely calibrated, replace measurements with hand-held calibrated sondes using the seep sampler whole water samples only.

### 4.2.3 User Interface

#### *4.2.3.1 Alarms*

A low flow alarm sounds when the p3SFS system's "pump is running at high speeds," but no alarm warns that measured flow is outside of the user-defined goal flow, regardless of pump speed.

**Recommendation:** Add alarms to the p3SFS, including indicating overly high or low sample flow. The p3SFS's alarms should be made more noticeable by using a rapid blinking feature since sound-based alarms are lost in the background noise of the engine room.

#### *4.2.3.2 p3SFS "Cleanability" and Guidance*

During *Project 41012* implementation, GSI personnel noted that the flexible intake and return hoses suspended from the ceiling may pose a contamination threat. Internal surfaces in the p3SFS may also present contamination concerns.

**Recommendation:** Improve the p3SFS's cleaning methods and materials for surfaces exposed to sample water, such as hoses and internal surfaces, in order to ease TO ability to thoroughly clean them.

#### *4.2.3.3 Trend Screen*

In their current state, the axes on the trend screen of the p3SFS are not scalable and are difficult to understand without adequate axis labels.

**Recommendation:** Modify the usability of the trend screen on the p3SFS by labeling the axes.

#### *4.2.3.4 Installation Checklists*

Section 1.4.1 of the p3SFS manual recommends using an installation checklist if the system has not been used for one week. Though use of an installation checklist is valuable, one week is a short duration of time to have to recheck many of the items on the list. Also many of the issues would be caught by the p3SFS self tests.

**Recommendation:** Relax the inactive period requirement that would trigger installation checks of the p3SFS.

#### *4.2.3.5 Flow Rate Display*

Currently the p3SFS is programmed to display and record instantaneous flow rate to the nearest gallon per minute. The p3SFS controls flow and calculates the TC summary using more significant digits than are displayed or logged. During the tests, GSI found that flow rates displayed by the p3SFS controller screen did not change for long periods of time, which raised questions as to whether the flow sensing system was functioning properly.

**Recommendation:** Change the p3SFS display to record flow rate to the $1/10^{th}$ gpm so that variations in flow will appear to provide the user with assurance that the signal is live. This modification would also make validating the calculated summary data from the p3SFS more feasible because currently averages from the data log using whole numbers do not match well with system-reported averages because the data log does not record significant figures. A programming change to the p3SFS should be made to allow proportional flow control if a flow meter were installed onboard a test vessel.

# 5 CONCLUSION

GSI found both the ETV DSP and p3SFS to be feasible and promising approaches to shipboard validation of prospective BWMSs. However, several improvements in both the ETV DSP and the p3SFS must be made to achieve effective implementation over time, across ships, and across TOs. For example, the ETV DSP should provide guidance and set requirements around protecting TO staff health and safety during shipboard tests, including preventing personnel over-extension, and exposure to harmful substances and organisms. It should also require contingency planning around unplanned changes to ballast flow rates, and implications for sample/ballast flow proportionality. The protocol must define an acceptable proportionality envelope as a data quality objective. Another significant logistical matter for the TO and the ship is whether "whole tanks" need to be sampled on discharge or whether partial tanks are valid sources of discharge water (provided in both cases that all subject intake water has been sampled and retained without amendment).

Given resident toxicity of many harbors, GSI also recommends that the ETV DSP require a qualitative determination for WET of intake water to assure proper interpretation of WET outcomes relative to post-treatment discharge. The ETV DSP required threshold conditions were rarely fully met in the *Project 41012* TCs, though failure to meet some of these requirements may not warrant invalidation of entire TCs. Still, POM and POC requirements are more easily and thoroughly addressed in land-based testing.

In terms of the p3SFS, GSI recommends retooling the positions of the flow control valve and flow meters to achieve accurate flow meter readings and flow control; streamlining commissioning and operation, including provision of additional sample ports; improving filter sock construction; and enhancing drip and grab sample collection capacity. Software improvements are necessary to assure accurate temperature and turbidity data, digital card error reporting, and pause and resume capacity. The user interface would be improved by revised alarms, better p3SFS "cleanability" and guidance, a trend screen, installation checklists, and a flow-rate display.

The BWMS undergoing testing in *Project 41012* was a useful subject for the ETV DSP demonstration. The ETV DSP of this BWMS on the M/V Indian Harbor showed that the BWMS reduced live organism concentrations relative to those observed during ballast intake, but the treated ballast water discharged by the Indiana Harbor did not meet the USCG's March 2012 numerical standards for indicator organism concentrations. WET tests conducted according to protocols described here showed a significant reduction in *C. dubia* reproduction exposed to treated effluent and dilutions thereof, relative to controls. There were no reproduction effect detected in any other test organism, and no acute effects detected in any test organism. There were also measurable concentrations of sodium ion found in the treatment discharge from treated tanks.

In conclusion, the ETV DSP represents a strong starting point for a standard shipboard BWT verification protocol, but greater specificity and clarity in specific areas are needed to assure that TOs have sufficient guidance to implement the protocol effectively and to avoid expensive false starts or compromised outcomes.

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# APPENDIX A. p3SFS FLOW CONTROL/FLOW METER POST-EXPERIMENT PROBLEM DIAGNOSIS

After completing the IH-based testing reported here as part of USCG RDC Project No. 41012 titled *Shipboard Approval Tests of Ballast Water Treatment Systems in Freshwaters*, GSI planned to undertake a land-based empirical comparison test of the p3SFS *vs*. GSI Ship Discharge Monitoring System, using the GSI land-based sampling system as a "control", at the GSI land-based testing facility. The purpose was to validate p3SFS performance under controlled circumstances against previously validated sampling approaches[36]. Prior to collecting biological samples, GSI set out to calibrate and validate performance of the flow meters of both ships sampling systems subject to comparison. In calibrating and validating the p3SFS flow meters, GSI directed water through the GSI land-based facility piping system into the p3SFS and a 227 gallon graduated tank. The calibration plan involved the following procedure.

1. The sample system was set to a target flow rate;
2. The sample system was started-up; water was not collected until the sample system had reached and stabilized on its target flow rate;
3. Once stable, the discharge flow was channeled into a tank with known volume. The duration to fill the tank was timed as well as the totalizer reading recorded at the start and end of the tank;
4. The flow meter reported volume was compared to the actual tank volume; and
5. If the flow meter was off, a correction factor would be calculated and applied and the system would be rechecked starting with step 1.

The p3SFS calibration procedure required multiple repetitions of Step 5– the attempt to apply a correction factor – because the p3SFS flow meter continued to give inaccurate results. NRL sent a replacement flow meter of a similar make and GSI installed it and re-ran the calibration, but the same issue remained with the new flowmeter. Attempted troubleshooting methods are listed below:

1. Replacement of the flow meter;
2. Using the flow meters built in flow calibration;
3. Manual calculation of k factors;
4. Restoring manufactures recommended k factor;
5. Better grounding the flow meter;
6. Changing the p3SFS pump speed;
7. Applying more back pressure to the skid to better simulate its intended installation;
8. Cleaning of the flow meters contacts;
9. Checking and realigning flow meter in its port; and
10. Running at different flow rates.

[36] GSI (2014). *Test/Quality Assurance Plan: Empirical Comparison of GSI and NRL Shipboard Sampling Systems at the GSI Land-Based Testing Facility*. Washington, D.C.: Northeast-Midwest Institute.

Finally, it was concluded that the flow control valve upstream of the flow meter must be causing the inaccurate flow meter results. It is likely that when the control valve moved in response to changes in net porosity or other flow fluctuations, it caused turbulence in the flow meter. It would be possible to calibrate the flow meter for a single flow rate if the control valve were held in a single position. Unfortunately, the control valve must move to maintain a flow rate as the sample nets clog. As a result it is very unlikely that the p3SFS could maintain the appropriate flow rate while collecting samples. The comparison between the two sampling systems was abandoned pending redesign of the p3SFS. In addition, data from the USCG RDC Project No. 41012 titled *Shipboard Approval Tests of Ballast Water Treatment Systems in Freshwaters*, involving knowledge of flow (i.e. zooplankton concentrations) were invalidated.

# APPENDIX B. GSI TEST/QUALITY ASSURANCE PLAN (TQAP)

Double click to open the standalone file.

GREAT SHIPS INITIATIVE

GSI/QAQC/SB/TQAP/4
Issue Date: November 5, 2013
Page 1 of 52

## Test/Quality Assurance Plan (TQAP)

### TEST CYCLE 4 OF THE GSI EVALUATION OF THE ETV DRAFT PROTOCOL FOR THE VERIFICATION OF BALLAST WATER TREATMENT TECHNOLOGY IN SHIPBOARD INSTALLATIONS (VERSION 5.2)

### November 5, 2013

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# APPENDIX C. GSI QUALITY ASSURANCE PROJECT PLAN (QAPP) FOR SHIPBOARD TESTS

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GSI/QAQC/QAPP/SB/1
Revision 1: May 13, 2013
Page 1 of 56

## GREAT SHIPS INITIATIVE (GSI)
## QUALITY ASSURANCE PROJECT PLAN (QAPP)
## FOR SHIPBOARD TESTS

**REVISION 1**
**May 13, 2013**

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